

1 agggagaggc agtgacccgt aaggctgtgc tgc~~t~~gccc~~t~~ gt~~tg~~atggca
51 ggct~~t~~ggccc tgcagccagg cact~~g~~ccctg ctgt~~g~~tact cct~~g~~caa~~a~~gc
101 ccaggtgagc aac~~g~~aggact gc~~c~~tg~~c~~agg~~t~~ ggagaact~~g~~c acccag~~c~~gg
151 gggagcagtg ctggacc~~g~~cg cgca~~t~~cc~~g~~cg cagttggcc~~t~~ cct~~g~~acc~~g~~tc
201 arcagcaa~~a~~g gct~~g~~cagctt gaac~~t~~gc~~g~~tg ga~~g~~act~~c~~ac aggact~~a~~ca
251 c~~t~~ggggca~~a~~g aagaacatca c~~t~~g~~c~~tg~~t~~ga caccgact~~t~~g tgcaac~~g~~cca
301 gcggggccca tgcc~~t~~gc~~g~~ ccggct~~g~~cc~~t~~ ccat~~cc~~~~t~~gc gct~~g~~c~~cc~~ct
351 gcact~~c~~ggcc tgc~~t~~gc~~t~~g gggacc~~c~~ggc cagctatagg ctctgggggg
401 cccc~~g~~ct~~g~~ca gcccacac~~t~~g ggt~~g~~gg~~t~~gc cccaggcc~~t~~ t~~g~~gccac~~t~~c
451 ctac~~a~~gaac ctggccc~~g~~gt gggagc~~c~~ct~~t~~ cct~~g~~ttcc~~t~~ gaggcacatc
501 craacg~~c~~a~~a~~g tttgac~~c~~aa~~g~~ tatgttt~~g~~ca ccccttttcc c~~n~~aacc~~c~~tg
551 ac~~t~~ttcc~~c~~at gggcc~~t~~tc caggatt~~cc~~n acc~~t~~ggc~~g~~aga tcagtttt~~g~~ag
601 tganacanat c~~g~~cn~~t~~gc~~g~~ agtggcc~~c~~ctc caaccn~~t~~tn t~~g~~ttgn~~t~~gt
651 tccatggccc agcattttcc acccttaacc ctgtgtt~~g~~ag gcacttttcc
701 cccc~~g~~agga~~a~~g cccccc~~t~~gc ccaccc~~c~~tt t~~g~~aaat~~g~~a g~~c~~cagg~~t~~tt~~g~~
751 g~~c~~cc~~t~~gg~~t~~g gcccc~~c~~gc~~g~~cc~~t~~ ccagcagg~~g~~gg acaggc~~t~~tc~~g~~ 2ggagg~~g~~ccc
801 agt~~g~~ttggc~~t~~ g~~g~~at~~g~~tt~~g~~gt ggac~~t~~g~~g~~ta g~~g~~act~~g~~gg~~g~~gg acaag~~g~~gt~~g~~
851 ac~~g~~ttgg~~g~~tc~~g~~ c~~g~~gg~~g~~at~~g~~tt cc~~g~~g~~g~~at~~g~~gg~~g~~gg~~g~~cc~~t~~gg~~g~~gg~~g~~aa
901 ggggccc~~g~~ggc~~t~~ c~~g~~ac~~a~~tt~~g~~ g~~g~~gg~~g~~ttcc~~t~~cc~~t~~ g~~g~~at~~g~~ggc~~g~~agc~~t~~g~~g~~ac~~a~~ag
951 c~~t~~agg~~g~~cc~~c~~tt~~g~~aa~~g~~ac~~t~~ c~~g~~ng~~g~~aa~~t~~ agcc~~a~~aaaaaa~~g~~aaaaaaa~~g~~

FIGURE 1A

M A V L L A L L M A G L A L Q P G T A L L C Y S C K A Q V S N E D C L Q V
E N C T Q L G E Q C W T A R I R A V G L L T V I S K G C S L N C V D D S
Q D Y Y V G K K N I T C C D T D L C N A S G A H A L Q P A A A I L A L L P A L
G L L W G P G Q L

FIGURE 1B

ATGAAGACAGTTTTTTATCTGCTGGCACCTACTTAGCCCTGCATCCAGGTGCTGCT
 1 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 TACTTCTGTCAAAAAAAATAGGACGACCAGTGGATGAATCGGGACGTAGGTCCACGACGA
 M K T V F F I L L A T Y L A L H P G A A -
 CTGCAGTGCTATTCATGCACAGCACAGATGAACAACAGAGACTGTCTGAATGTACAGAAC
 61 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 GACGTACGATAAGTACGTGTCGTCTACTTGTGTCGTACAGACTTACATGTCTTG
 L Q C Y S C T A Q M N N R D C L N V Q N -
 TGCAAGCCTGGACCAAGCACAGTTGCTTACATCGCGATCCGGGCCATTGGACTCGTGACA
 121 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 ACGTCGGACCTGGTCGTCAACGAAATGTAGCGCGTAGGCCCGTAACCTGAGCACTGT
 C S L D Q H S C F T S R I R A I G L V T -
 'GTTATCAGTAAGGGCTGCAGCTCACAGTGTGAGGATGACTCGGAGAACTACTATTGGC
 181 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 CAATAGTCATTCCGACGTGAGTGTACACTCCTACTGAGCCTTTGATGATAACCCG
 V I S K G C S S Q C E D D S E N Y Y L G -
 AAGAAGAACATCACGTGCTGCTACTCTGACCTGTGCAATGTCAACGGGGCCACACCCCTG
 241 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 TTCTTCTTGATGTGACGACGATGAGACTGGACACGTTACAGTTGCCCGGGTGTGGAC
 K K N I T C C Y S D L C N V N G A H T L -
 AAGCCACCCACCACCCCTGGGCTGCTGACCGTGCTGCAGCCTGTTGCTGGGCTCC
 301 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 TTCCGTGGTGGTGGACCCGACGACTGGCACGAGACGTCGGACAAACGACACCCGAGG
 K P P T T L G L L T V L C S L L L W G S -
 AGCCGTCTGAGGCTCTGGGAGAGCCTACCATAGCCCATTGTGAAGGGATGAGCTGCAC
 361 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 TCGGCAGACATCCGAGACCCCTCTGGATGGTATCGGCTAACACTCCCTACTCGACGTG
 S R L *
 TCCACCCCCACACAGG
 421 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 AGGTGGGGTGGGGTGTGTCC 441

FIGURE 2

1 M E K I F F P V V D E A N L L G V F R A S S NSCA-2
1 M K A V I L L A L L M A G L A L O P G T A NPSCA
1 M K I V L F L L H A T Y L A L H P G A A mPSCA

21 L M C F S C L N Q K S N L Y C E K P T I
21 L L C Y S C K A Q V S N S D C L Q V E N
21 L Q C Y S C T A Q M N N R D C L N V Q N

41 C S D Q Q N Y C V T V S A S X G I G N L
41 C T Q L G E Q C W T A R I R A V G L L T
41 C S L D Q H S C F T S R I R A I G L V T

61 V T F G H S L S K T C S P A C P I P E G
61 V - - - - I S K G C S L N C V D D S Q
61 V - - - - I S K G C S I S Q C E D D S E

81 V N V G V A S M G I S C C Q S F L C N S F
76 D Y Y V G K K - N L T C C D T D L C L N S A
76 N Y Y L G K K - N Y I T T C C Y S D L C N V

101 S A A D G G L R A S V T E L G A G I L I
95 S F G A H A L Q P A A A I L A L L P A E G
95 N G A H T L K P P T T L G L E T V L C S

121 S L T P A L L R F G P
115 L L L W G P G O L -
115 L L L W G S S S R L -

FIGURE 3

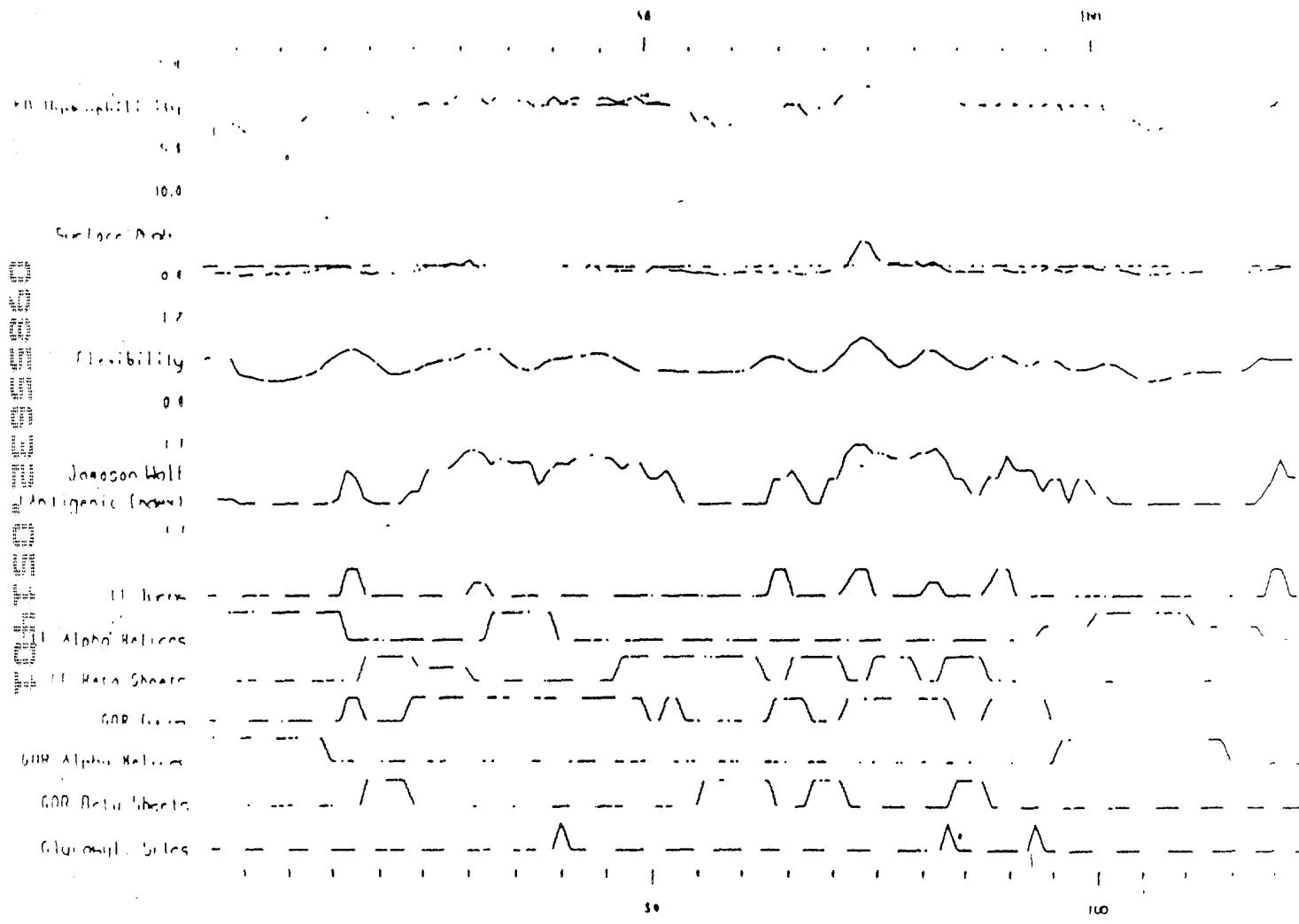


FIGURE 4

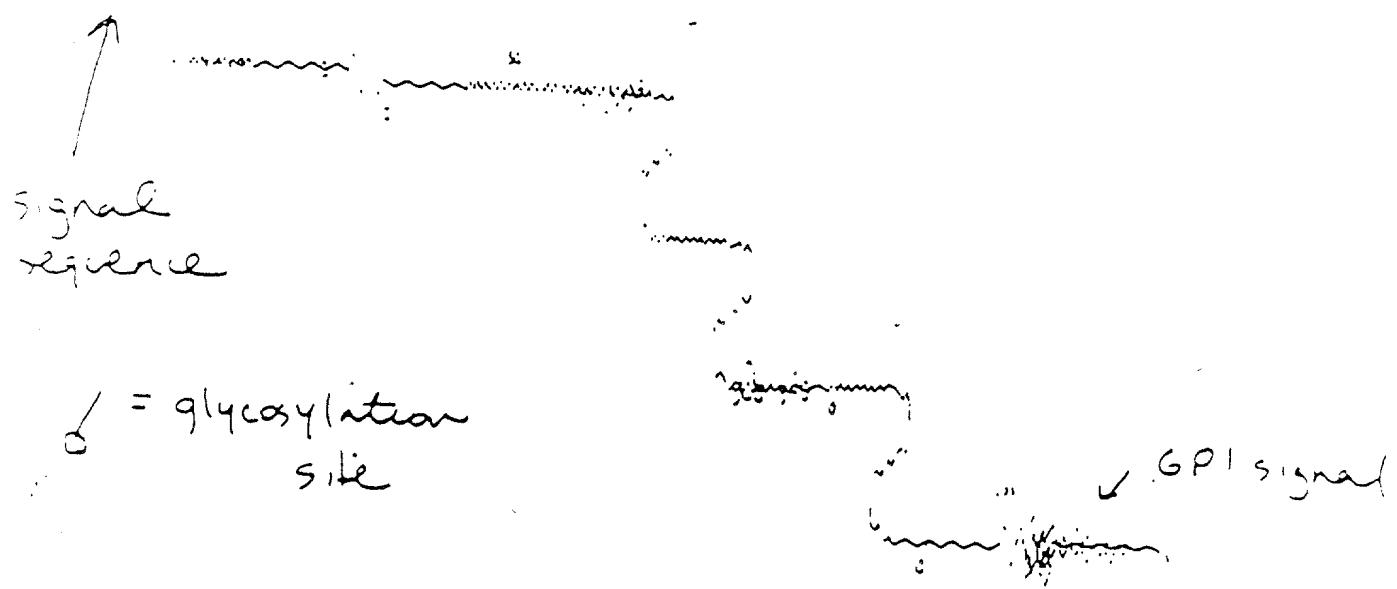
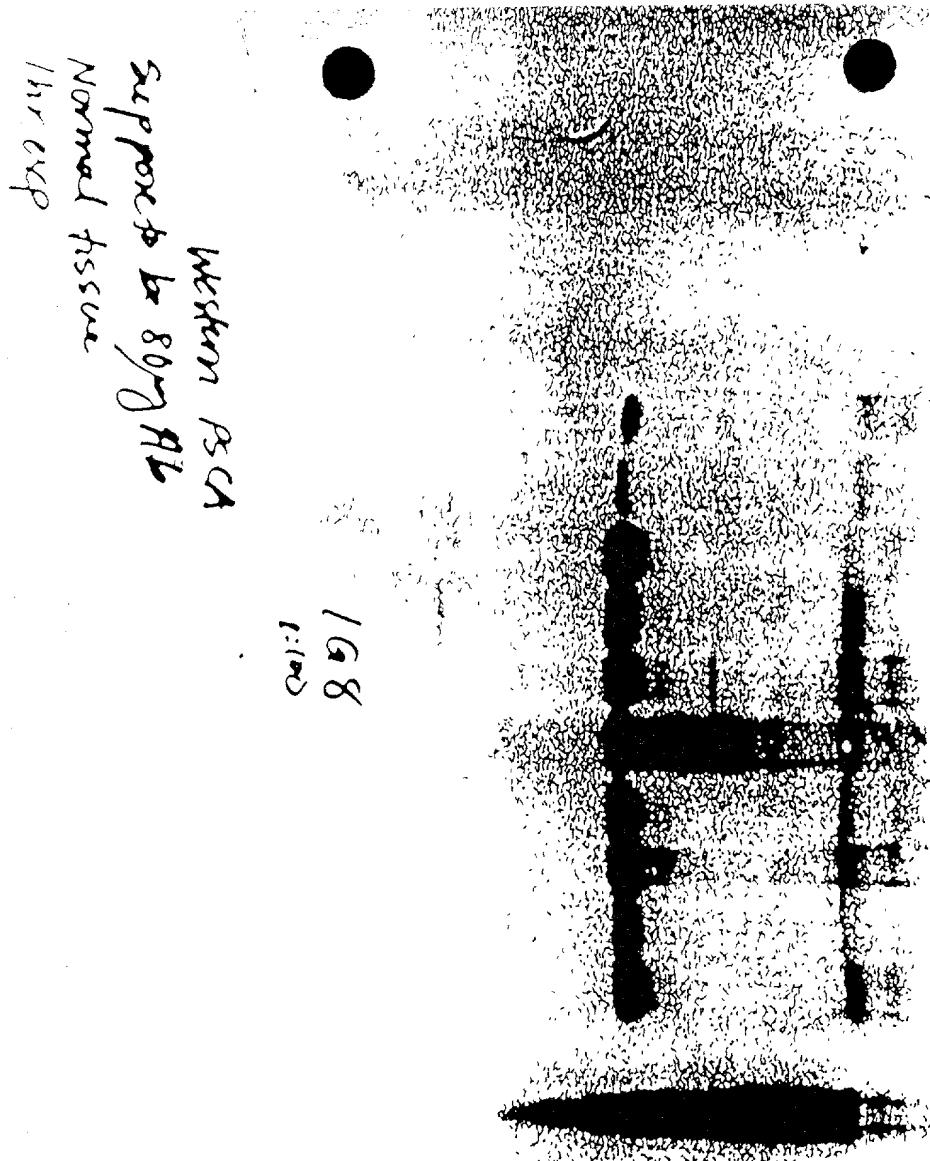


FIGURE 5



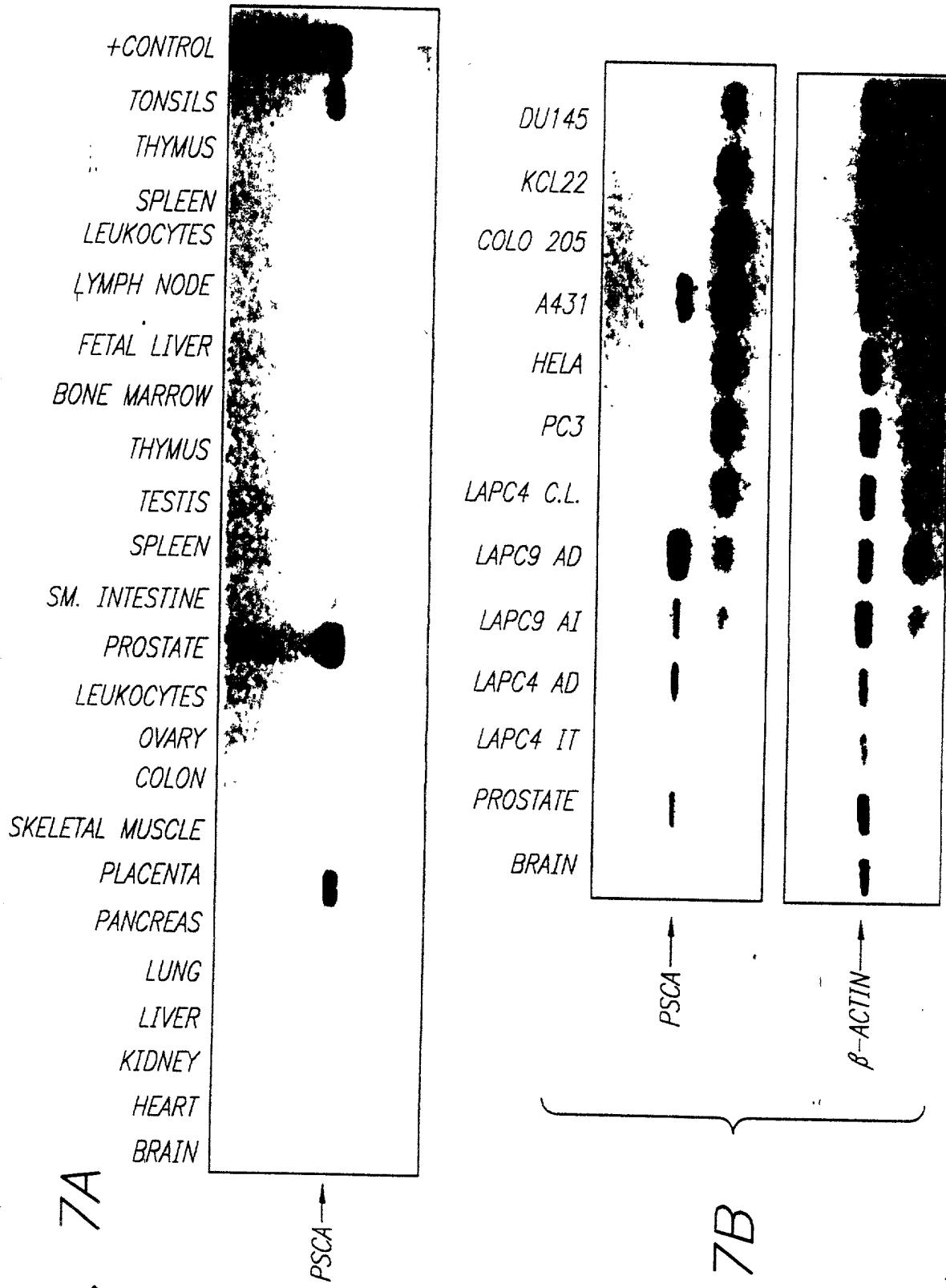
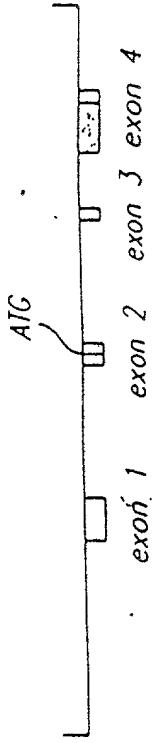


FIG. 7A

FIG. 7B

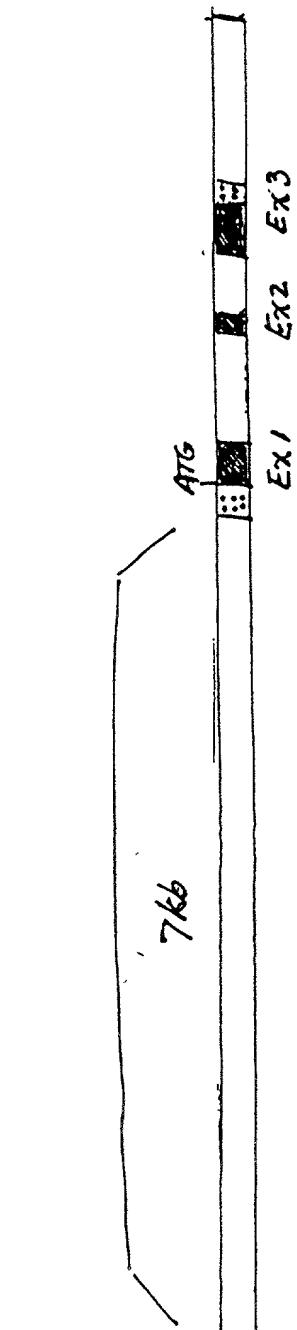
Legend: untranslated region of pSCA
 translated region of pSCA

FIG. 8A



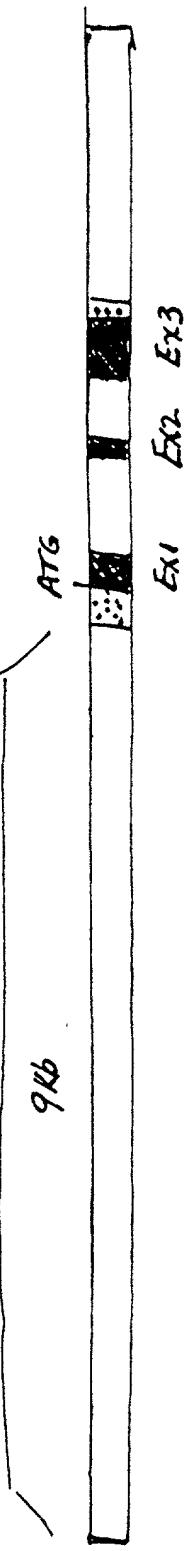
mouse pSCA

FIG. 8B



mouse pSCA

FIG. 8C



human pSCA

FIGURE 8

PSCA / PSA Expression in Benign Prostate vs. Prostate Cancer Xenograft

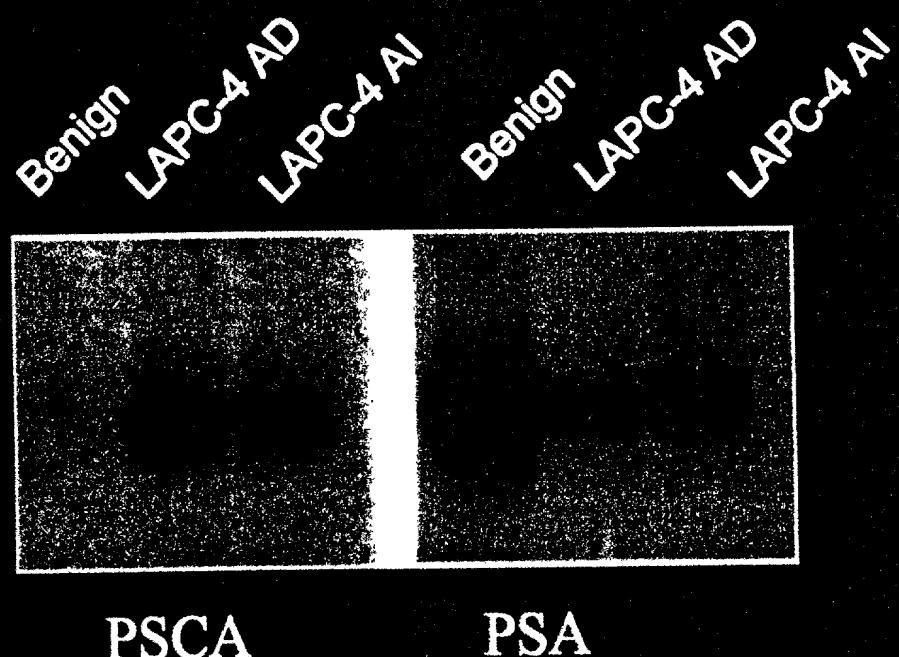


FIGURE 9A

FIG. 9B

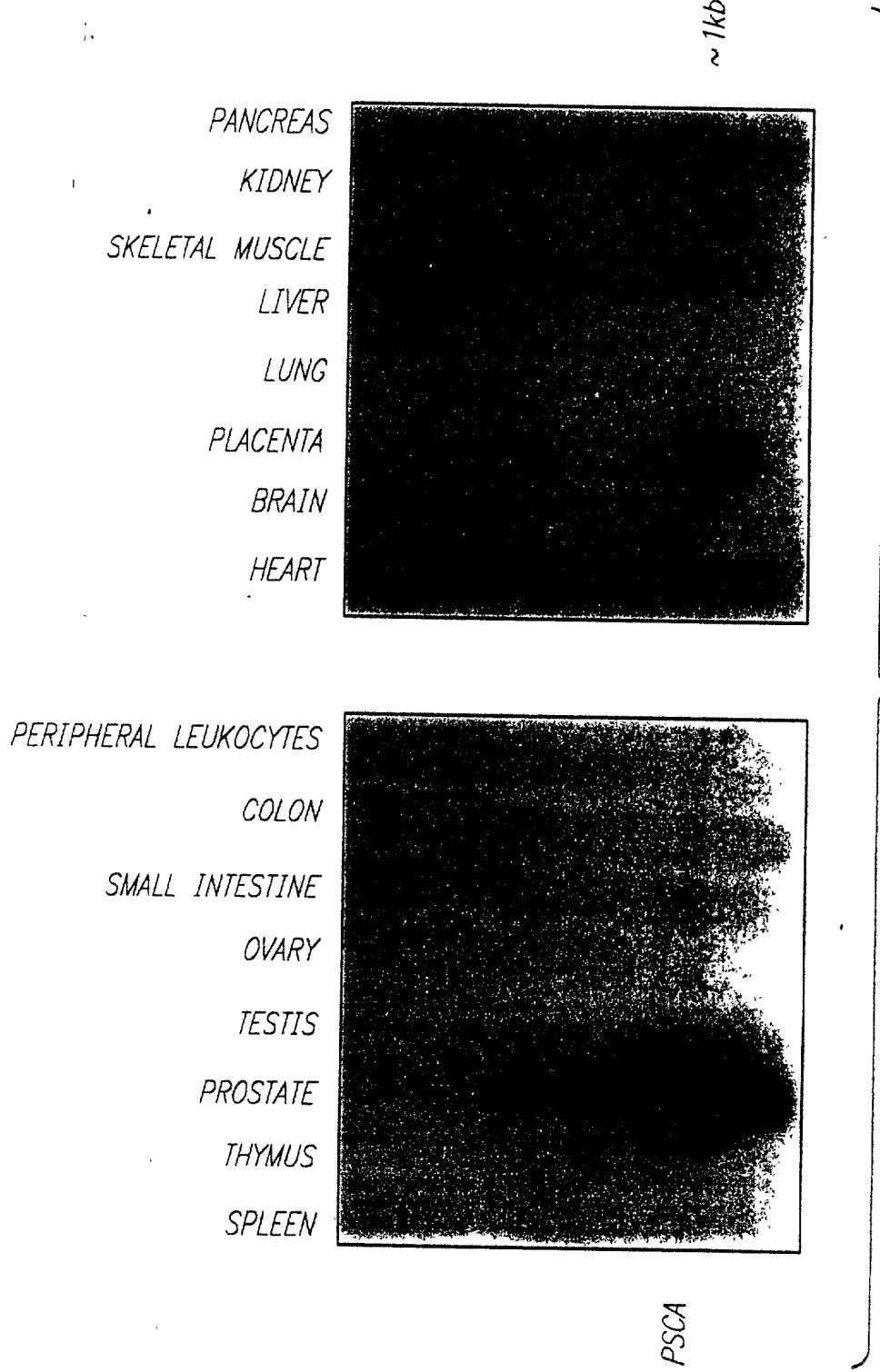


FIG. 10-1

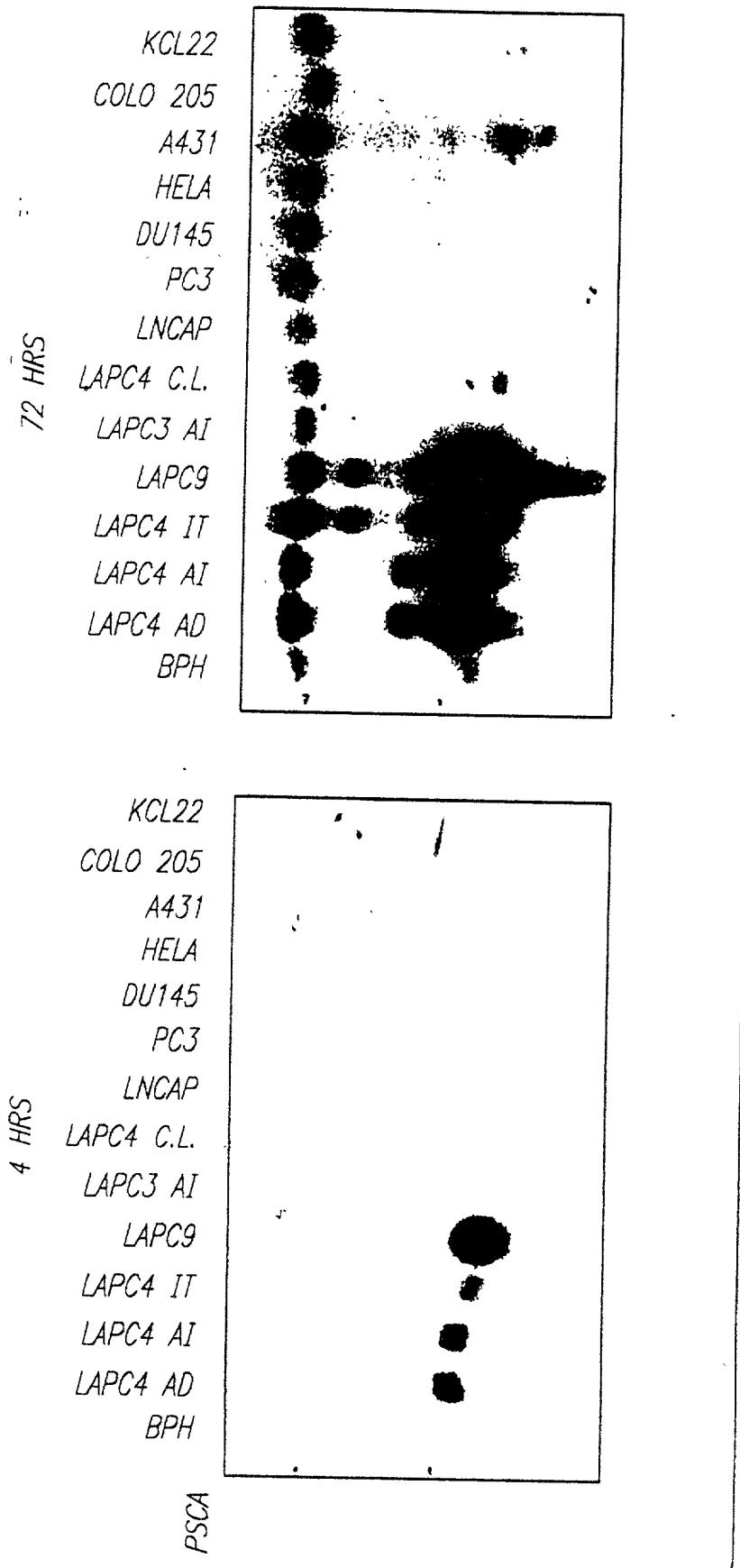


FIG. 10-2

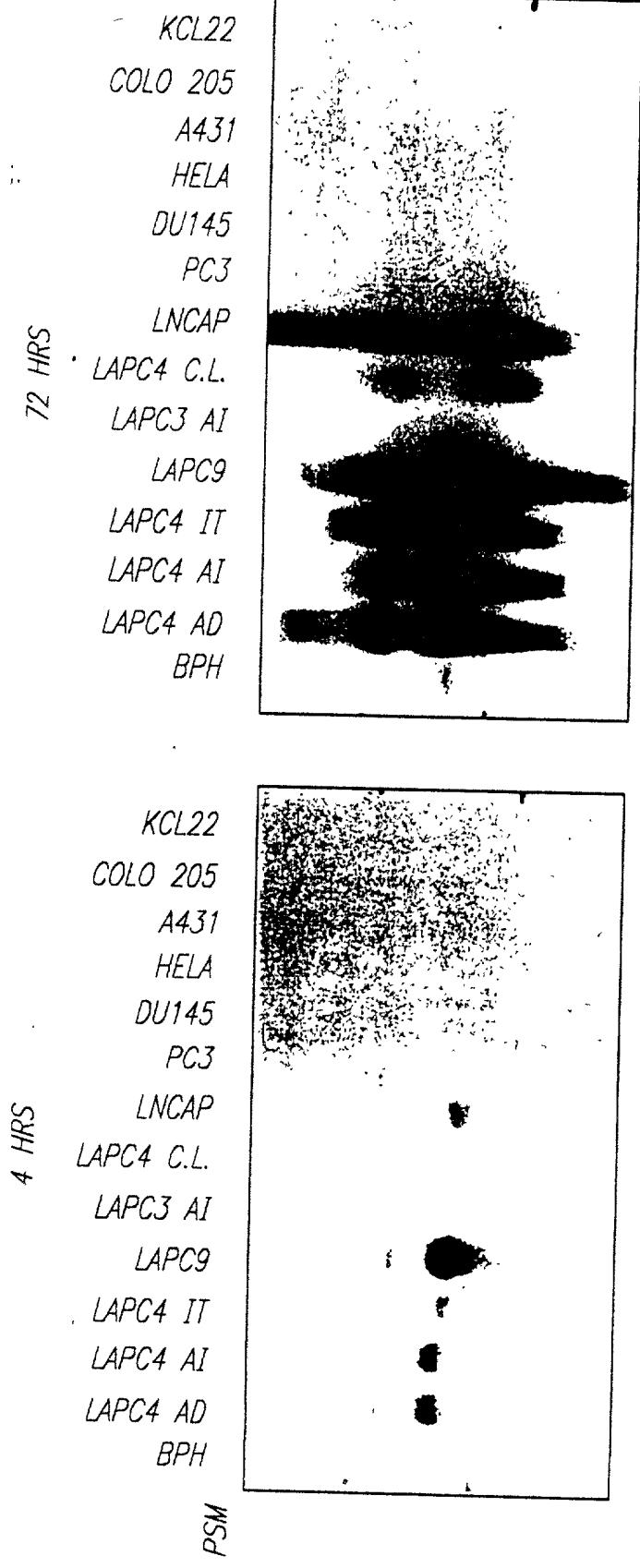


FIG. 10-3

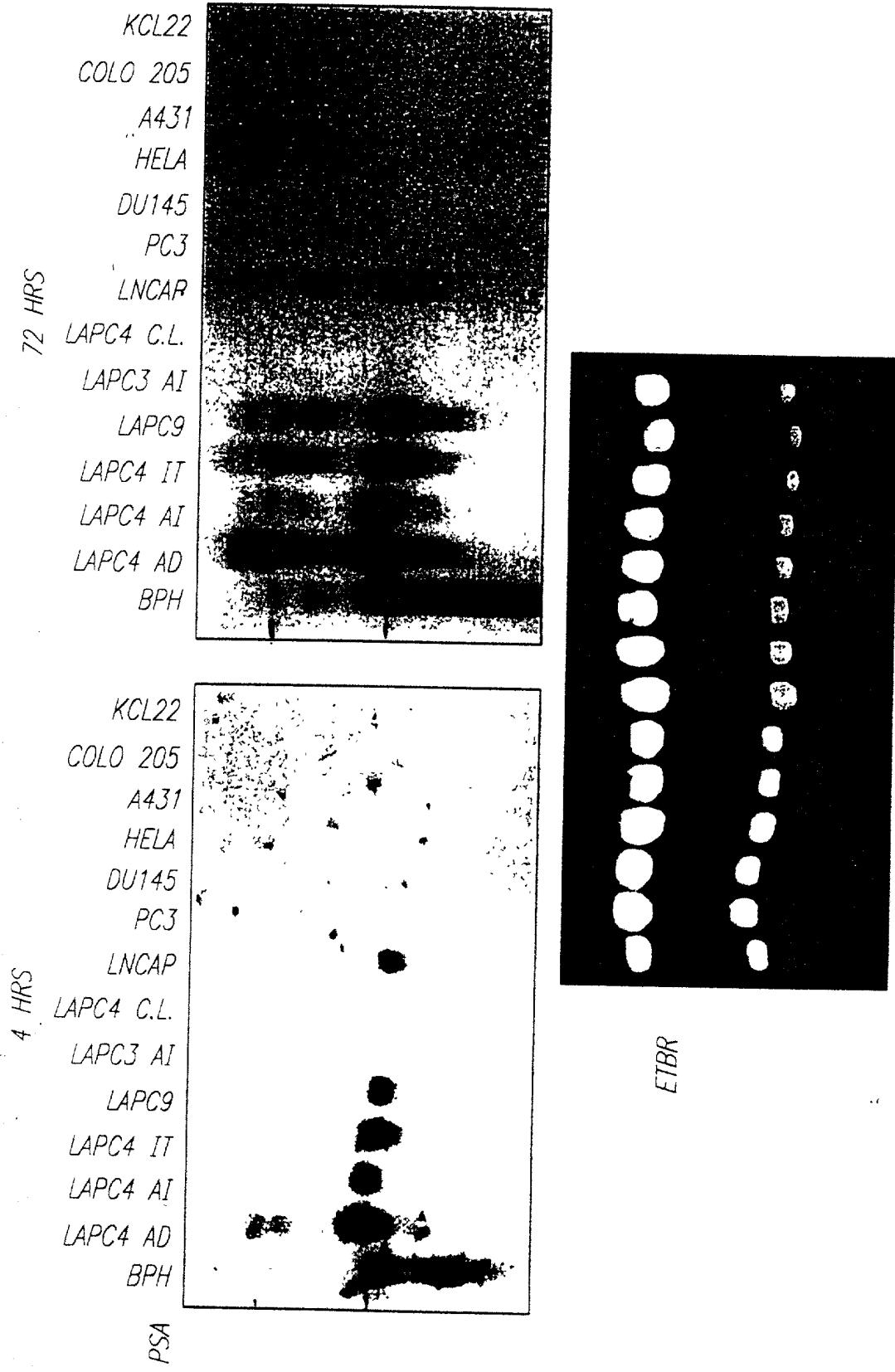
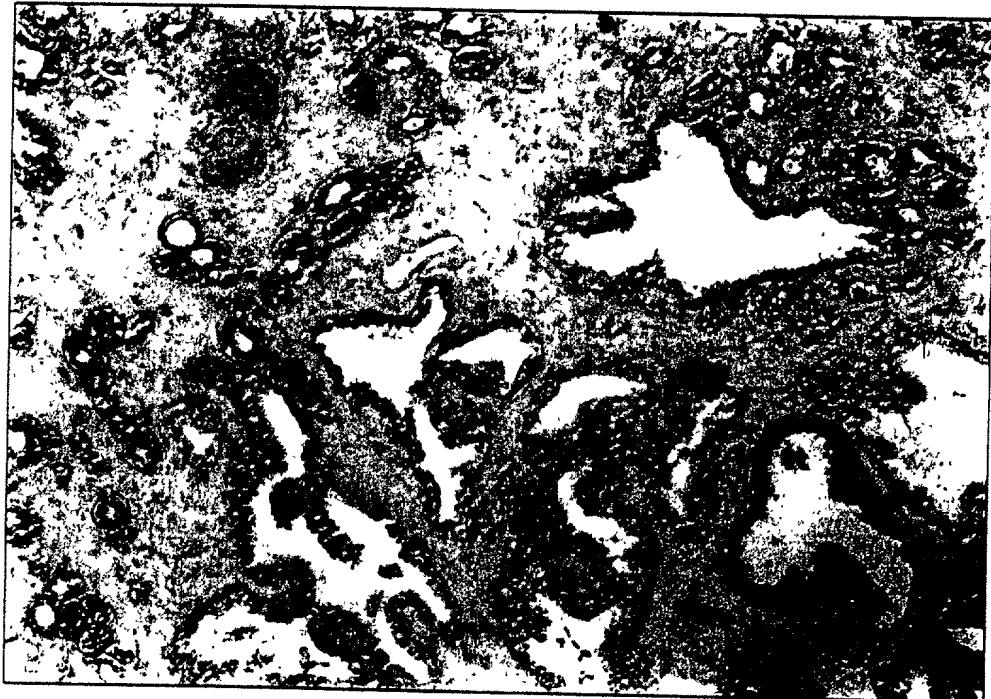


FIG. 11A



FIG. 11B



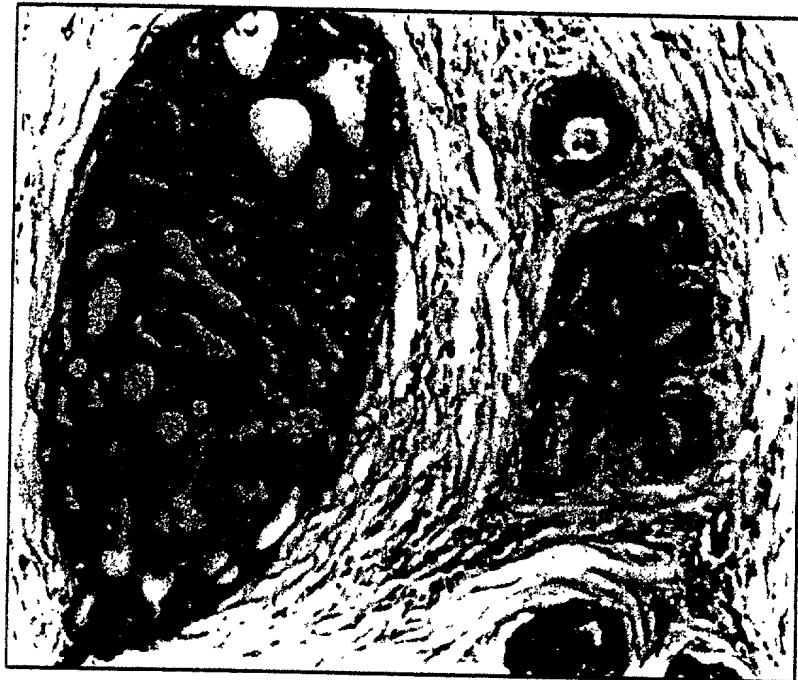
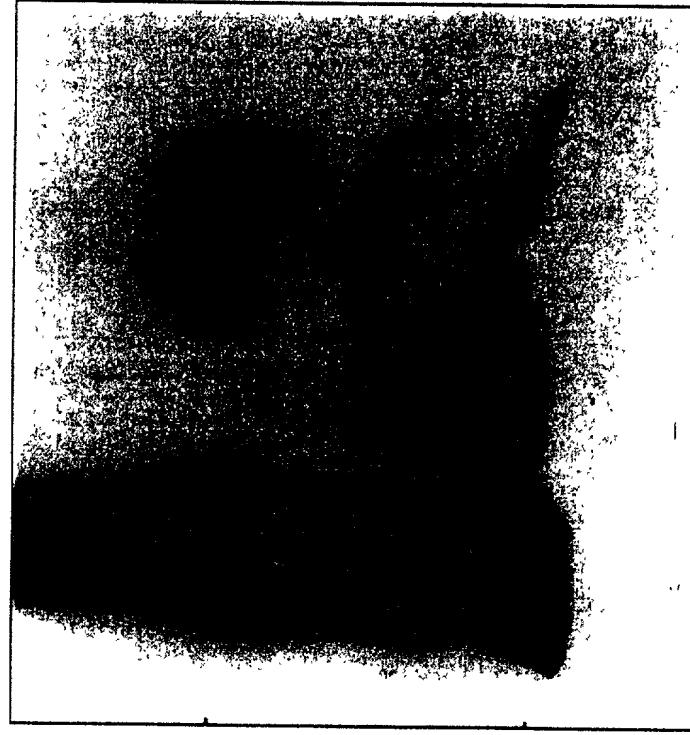


FIG. 11C

FIG. 12A

SECRETED
CELL ASSOCIATED
O GLYCOSIDASE
N GLYCOSIDASE F
CONTROL



~24kDa →
~10kDa →

FIG. 12B

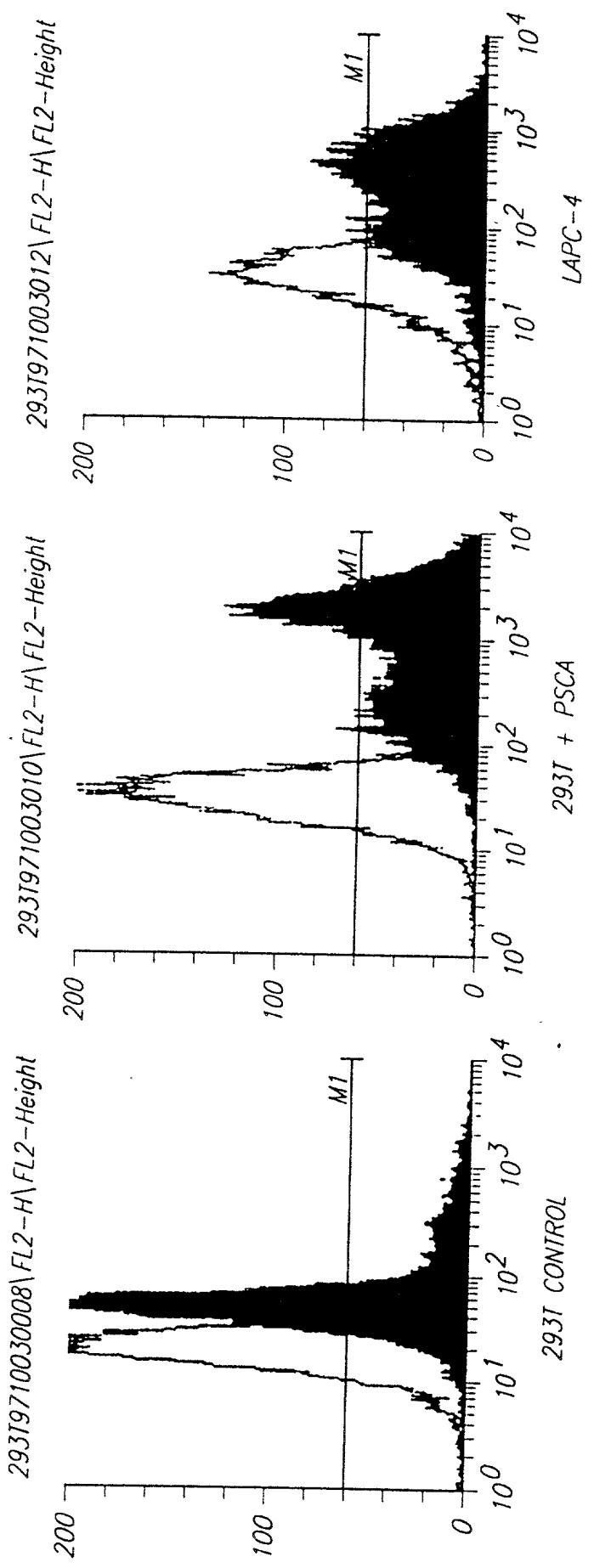
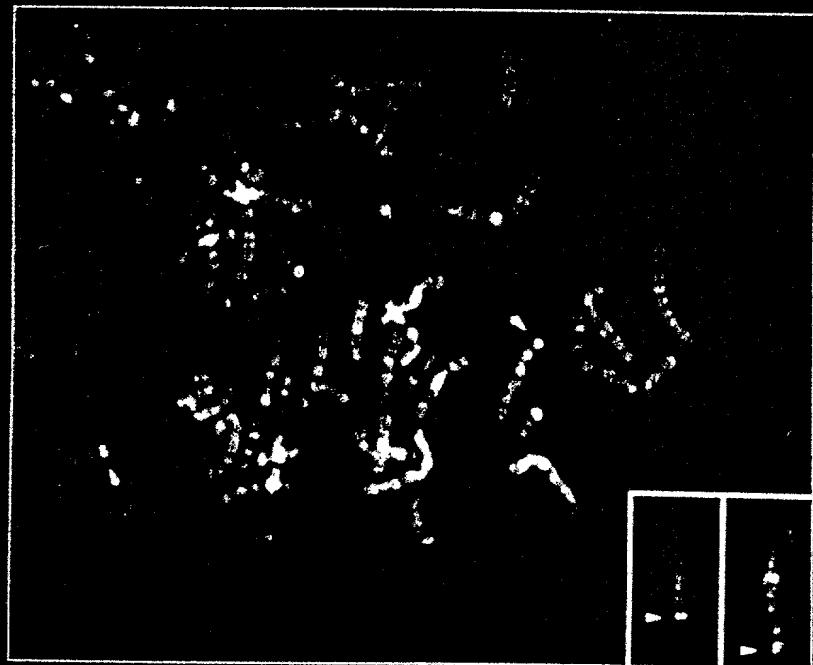


FIGURE 12C

PSCA Maps to Chromosome 8q24.2



Fluorescent
in Situ Hybridization
Analysis of PSCA

FIGURE 13

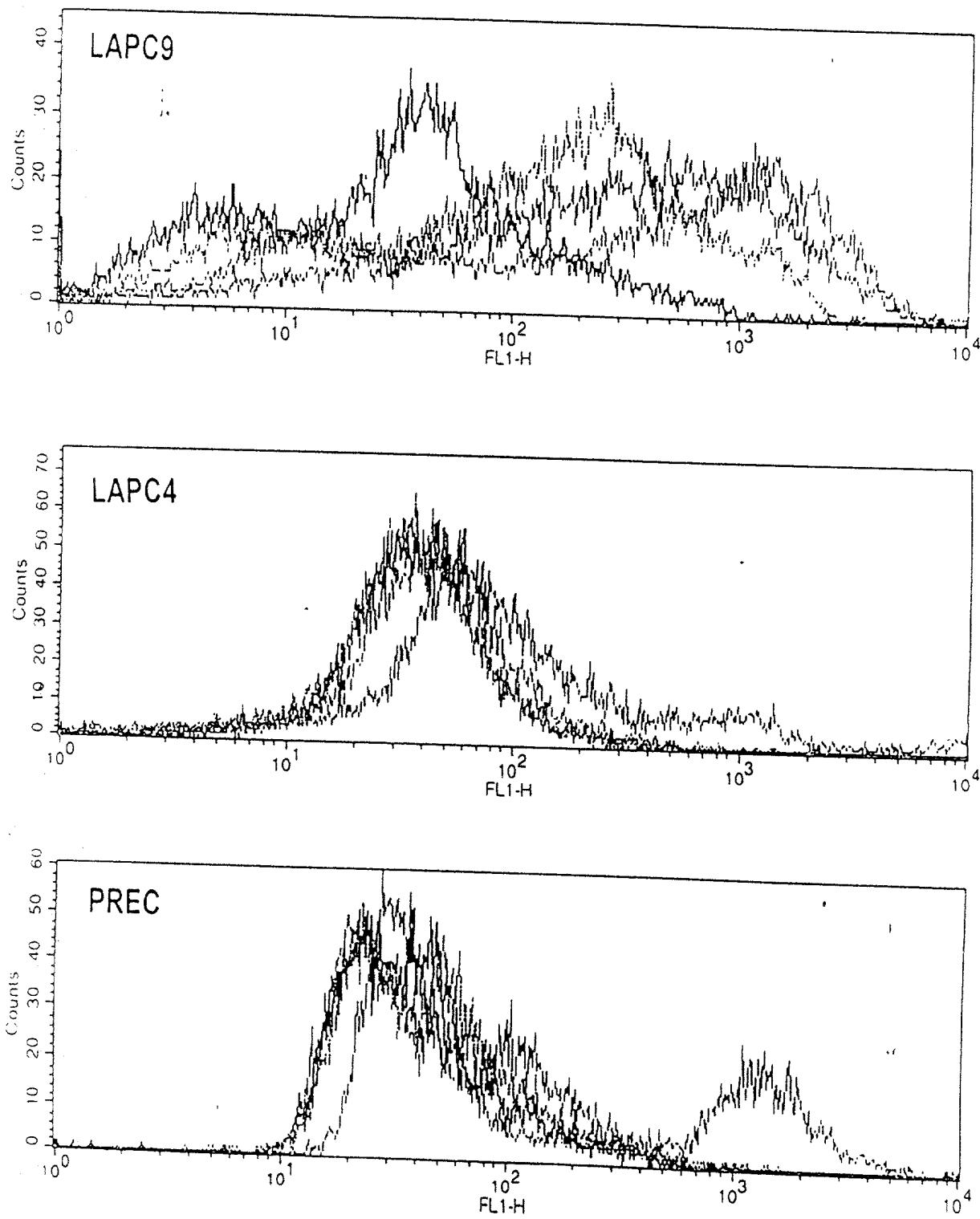


FIGURE 14

A

Epitope map

mAb	Isotype	EL (18-98)	N (2-50)	M (46-109)	C (85-123)
1G8	IgG1	2.039	0.007	0.628	0.000
2H9	IgG1	1.318	0.863	0.032	0.021
3C5	IgG2a	2.893	1.965	0.016	0.005
3E6	IgG3	0.328	0.024	0.069	0.370
4A10	IgG2a	2.039	1.315	0.000	0.014
2A2	IgG2a	1.366	0.733	0.010	0.003
3G3	IgG2a	2.805	1.731	0.004	0.000

B

1G8 2A2 3C5



FIGURE 15

Prostate Stem Cell Antigen (PSCA) is a GPI-anchored Protein

1	A F S N P	I D L A I A	L	hSCA-2
1	A D E H L A	T S M A	D I A D I P G I I A	hPSCA
1	T D K E D	I D E A I	I D A I I P G I I A	mPSCA
21	M G E S C	C D V Q	S N * C L	
21	G C S C X	A Q	S N * E D C L V	N *
21	Q C M S C T	A Q	M N P D C L I V	N *
41	G S	C T	A S A	
41	G S	C T R I R A	G L T	
41	G S	C T R I R A	G L T	
61	V	I S K G C S	C	
61	V	I S K G C S	C D D S	
61	V	I S K G C S	C D D S	
81	V N I S S M G I	C C	S F L C N *	
76	D Y Y G K W K	N I T C C	T D L C N *	
76	D Y Y G K W K	N I T C C	S D L C N *	
101	S A A N D G S R A	L L	L L	
95	S A A N D G S R A	L A L	L	
95	N T D K P	L L	L	
121	S T D P A			
115	S T D P A			
115	S T D P A			

(Reiter, R.E., et al., 1997, *PNAS*)

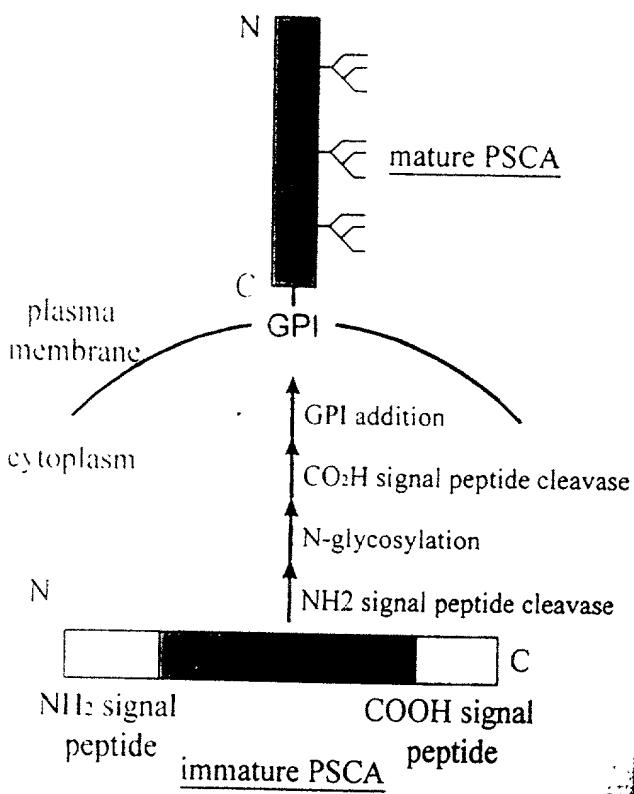
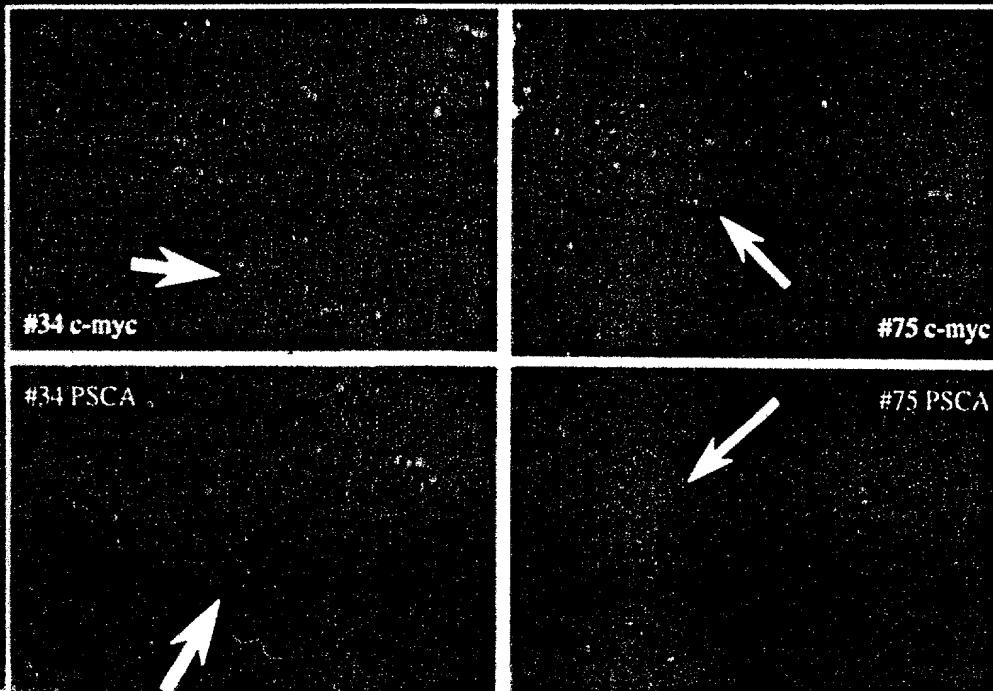


FIGURE 16

FISH Analysis of PSCA and c-myc in Prostate Cancer

Gain Chromosome 8

Amplification



R. Jenkins

FIGURE 17

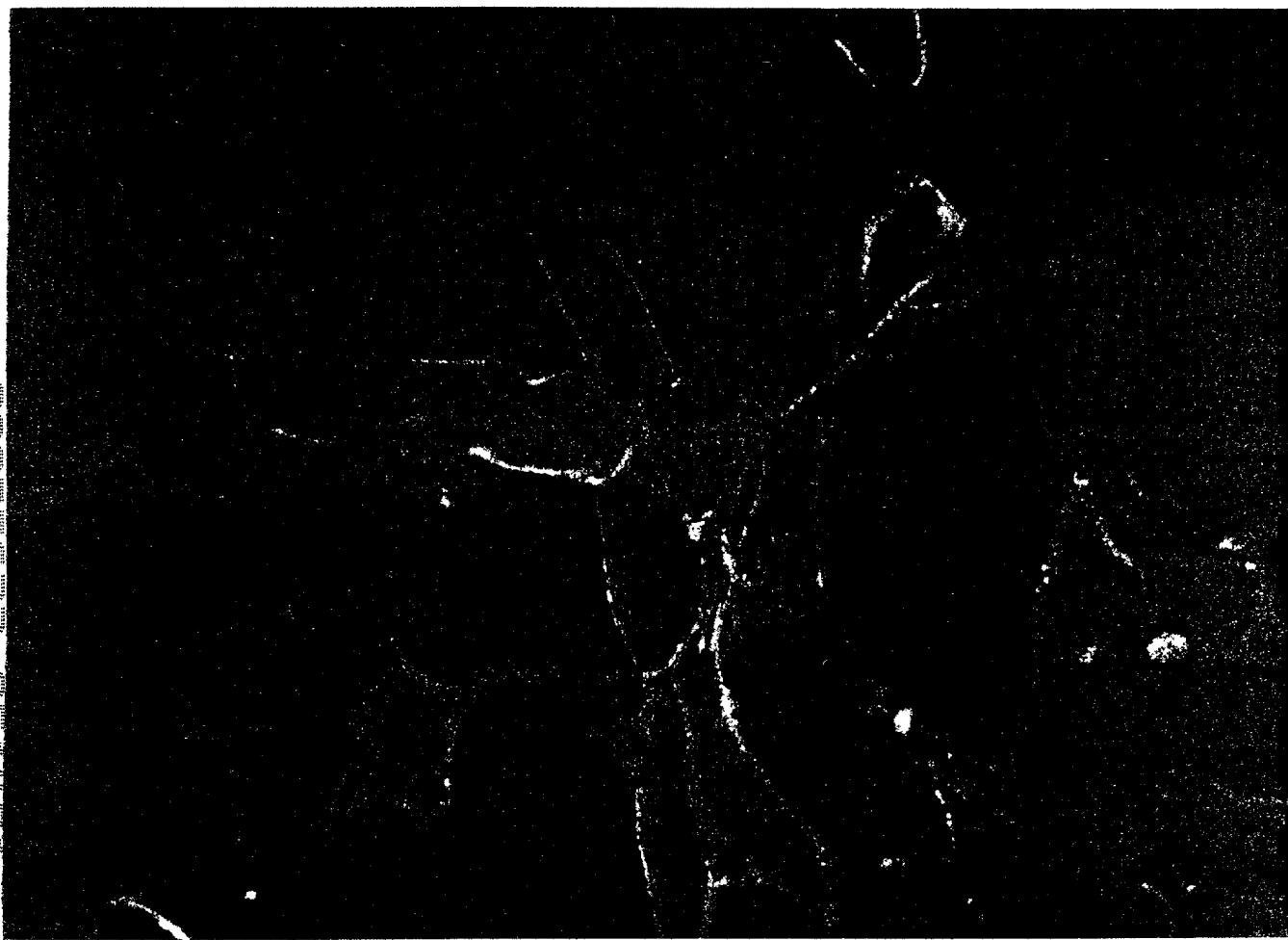


FIGURE 18

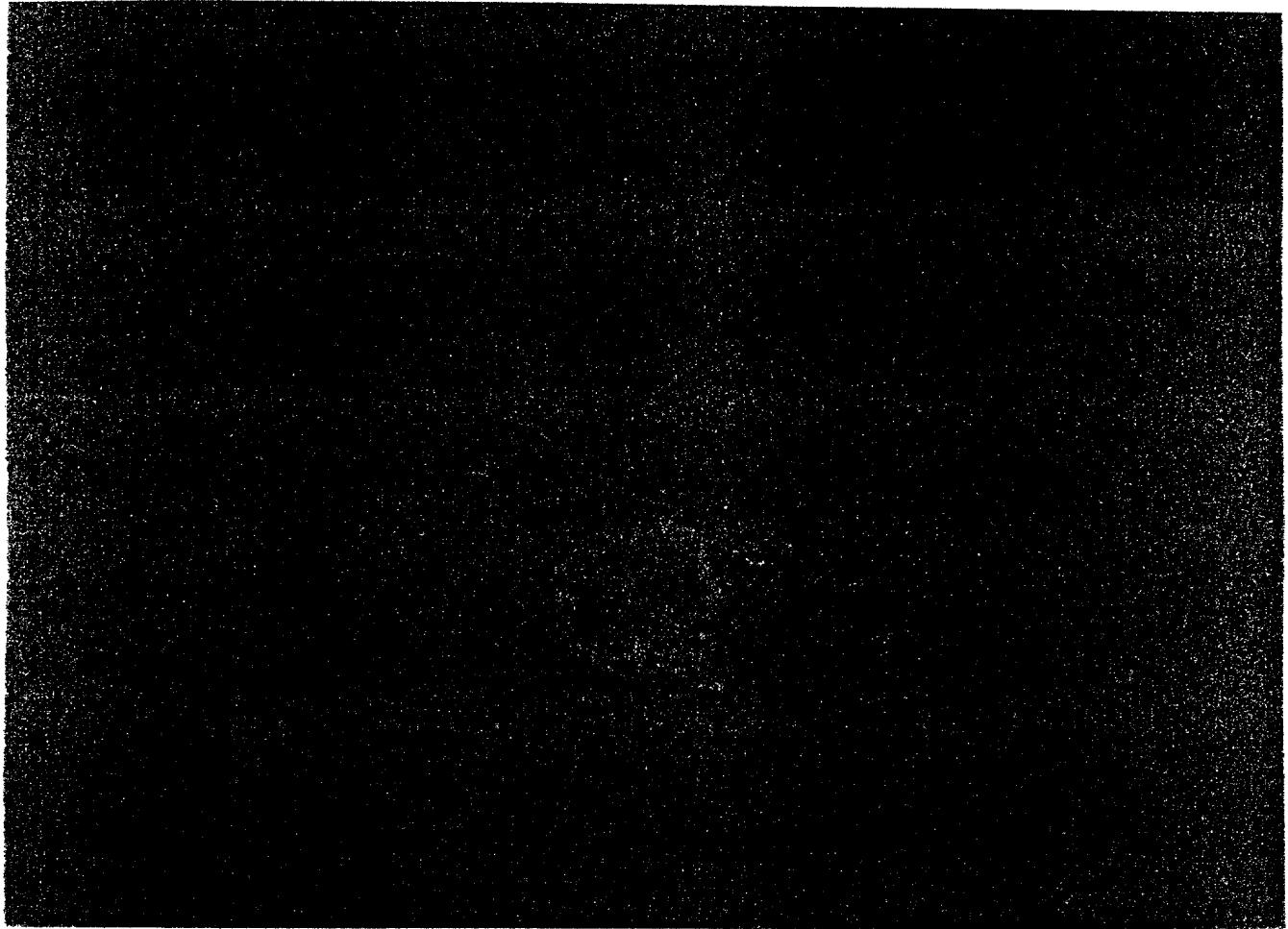


FIGURE 19



FIGURE 20

PSCA Immunostaining of Primary Tumors



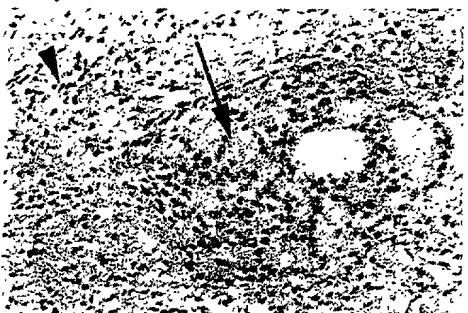
patient 1: mAb 1G8



patient 3: mAb 1G8



patient 2: mAb 1G8



patient 4: mAb 3E6

FIGURE 21



FIGURE 22



FIGURE 23



FIGURE 24

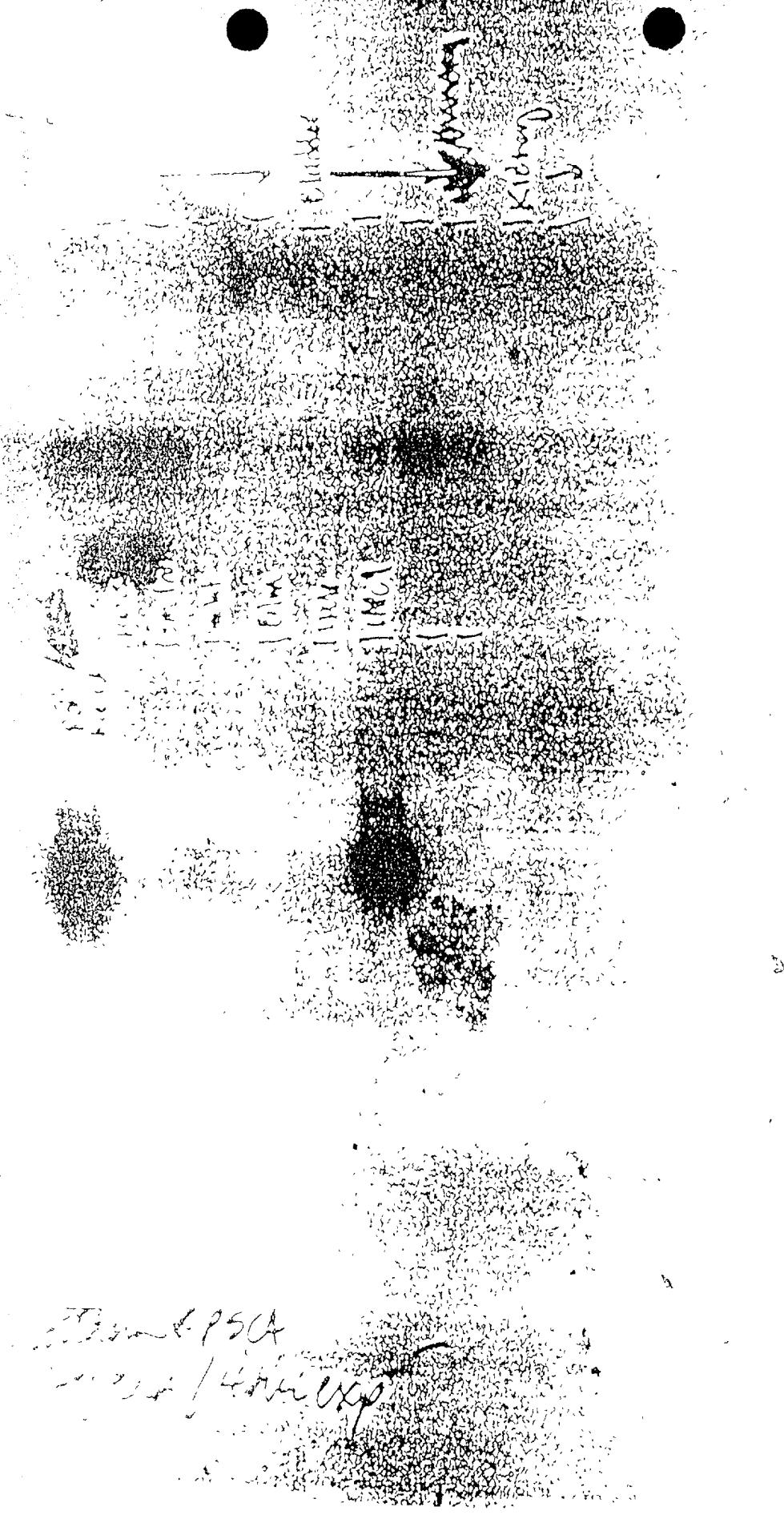


FIGURE 25

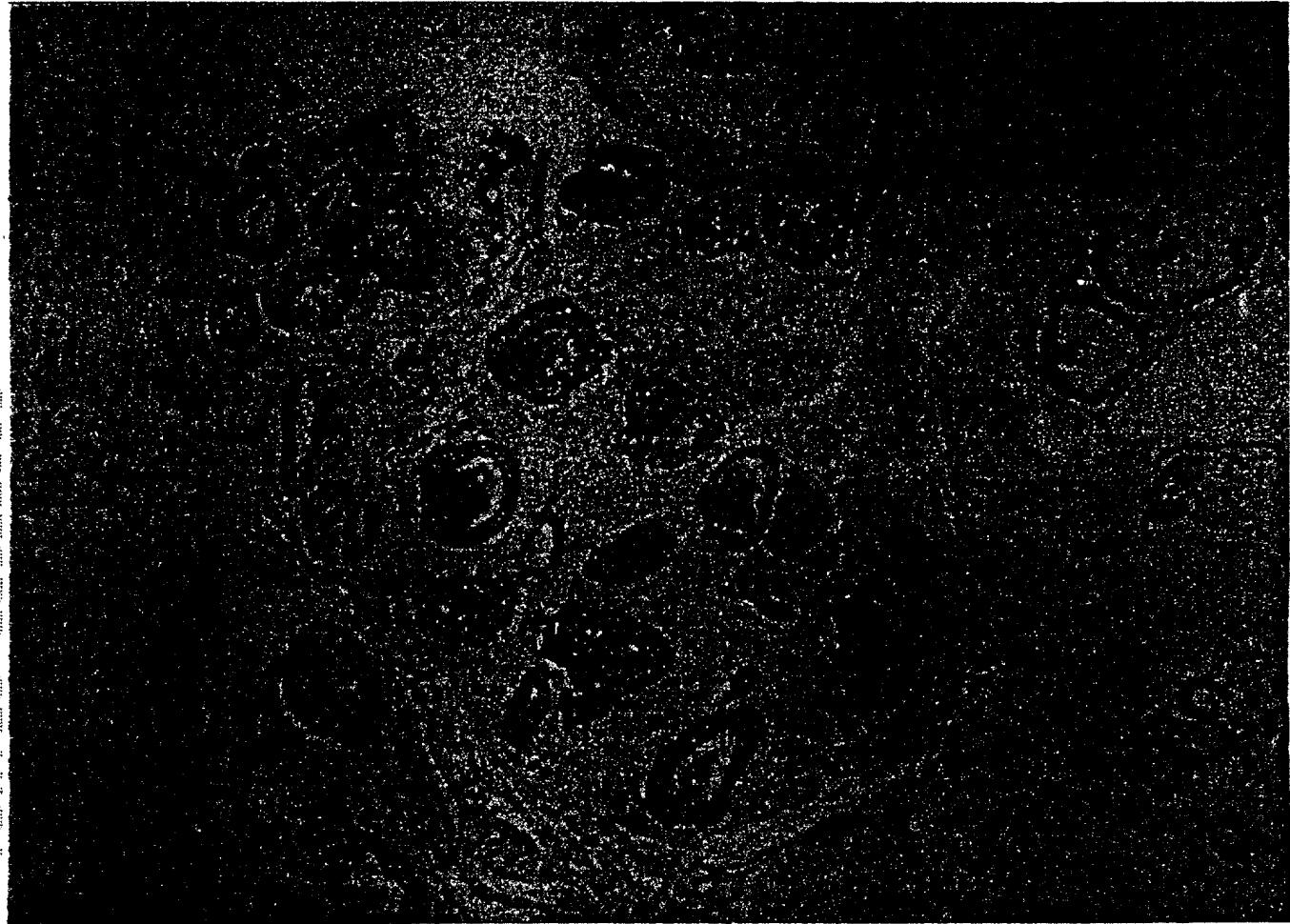
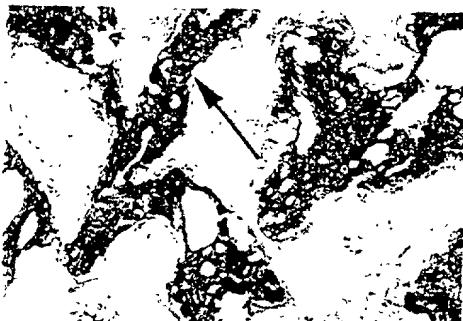
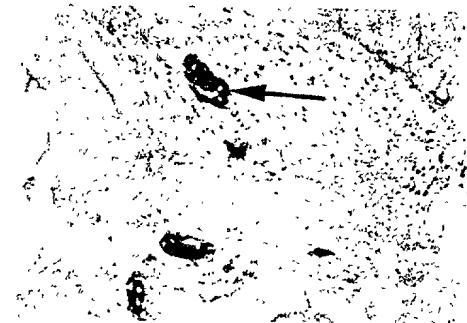
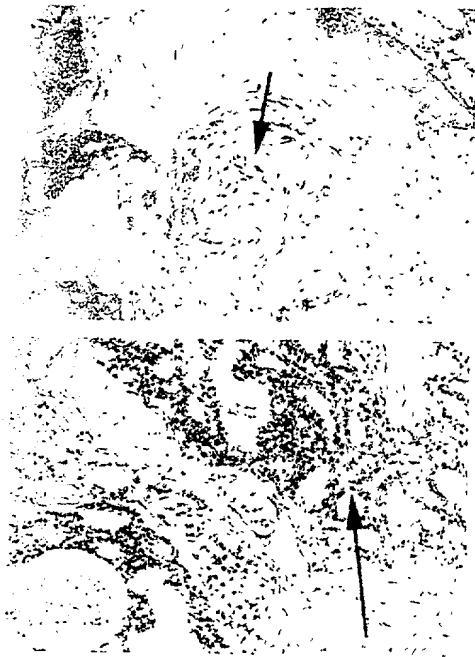


FIGURE 26



FIGURE 27

PSCA Immunostaining of Bony Metastases



Patient 5: H and E
and mAb 1G8

Patient 4: H and E
and mAb 3E6

FIGURE 28

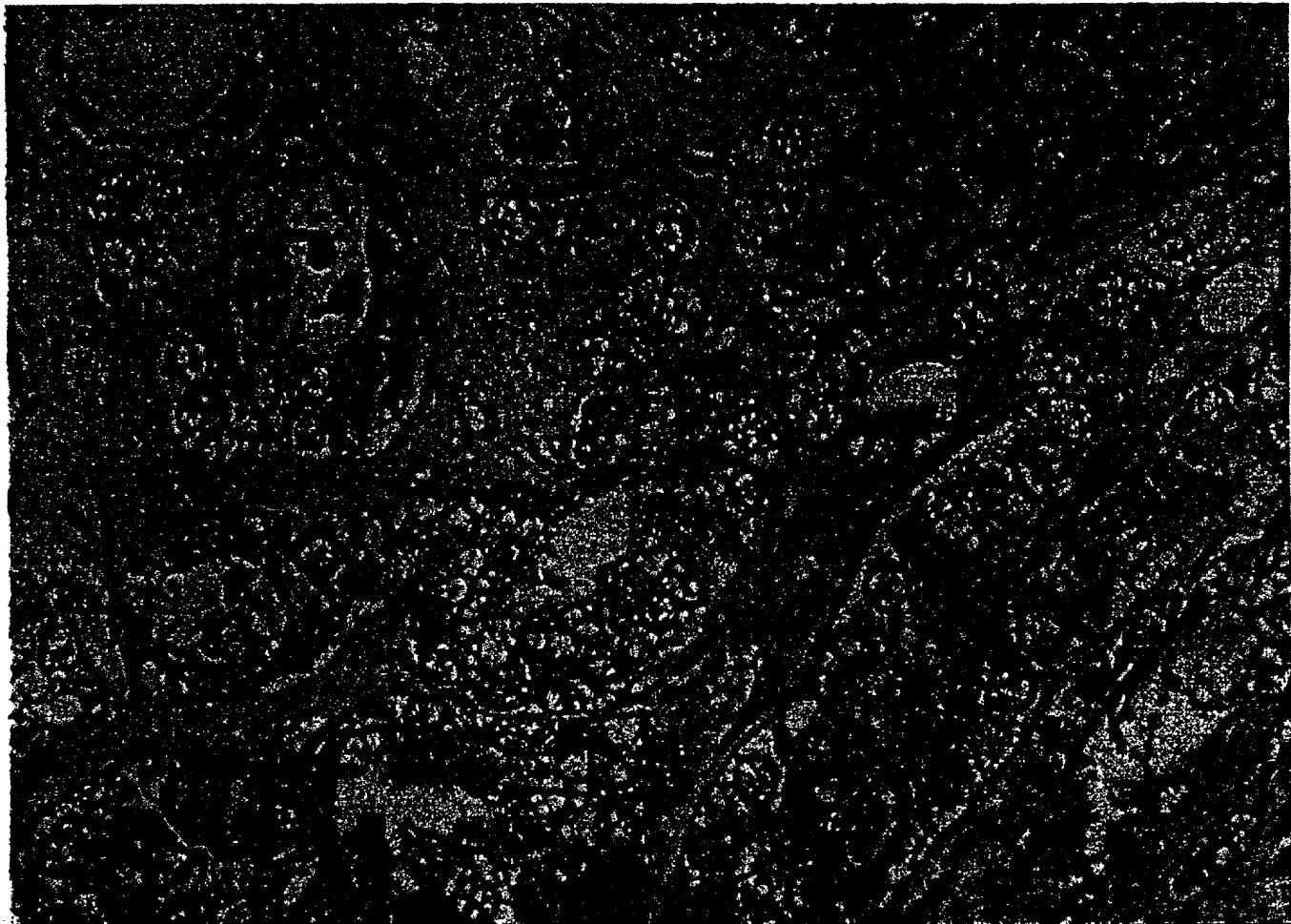


FIGURE 29

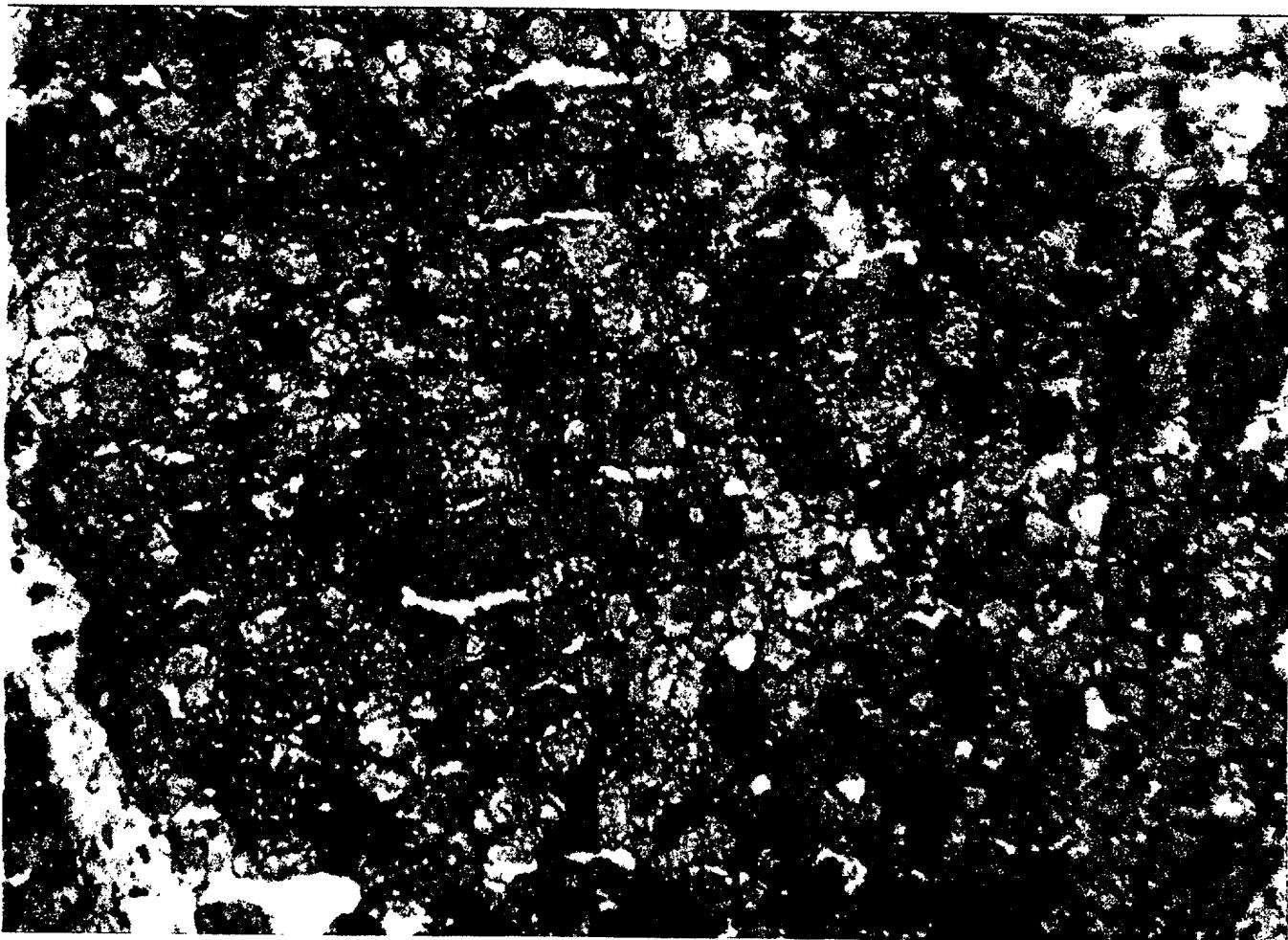


FIGURE 30

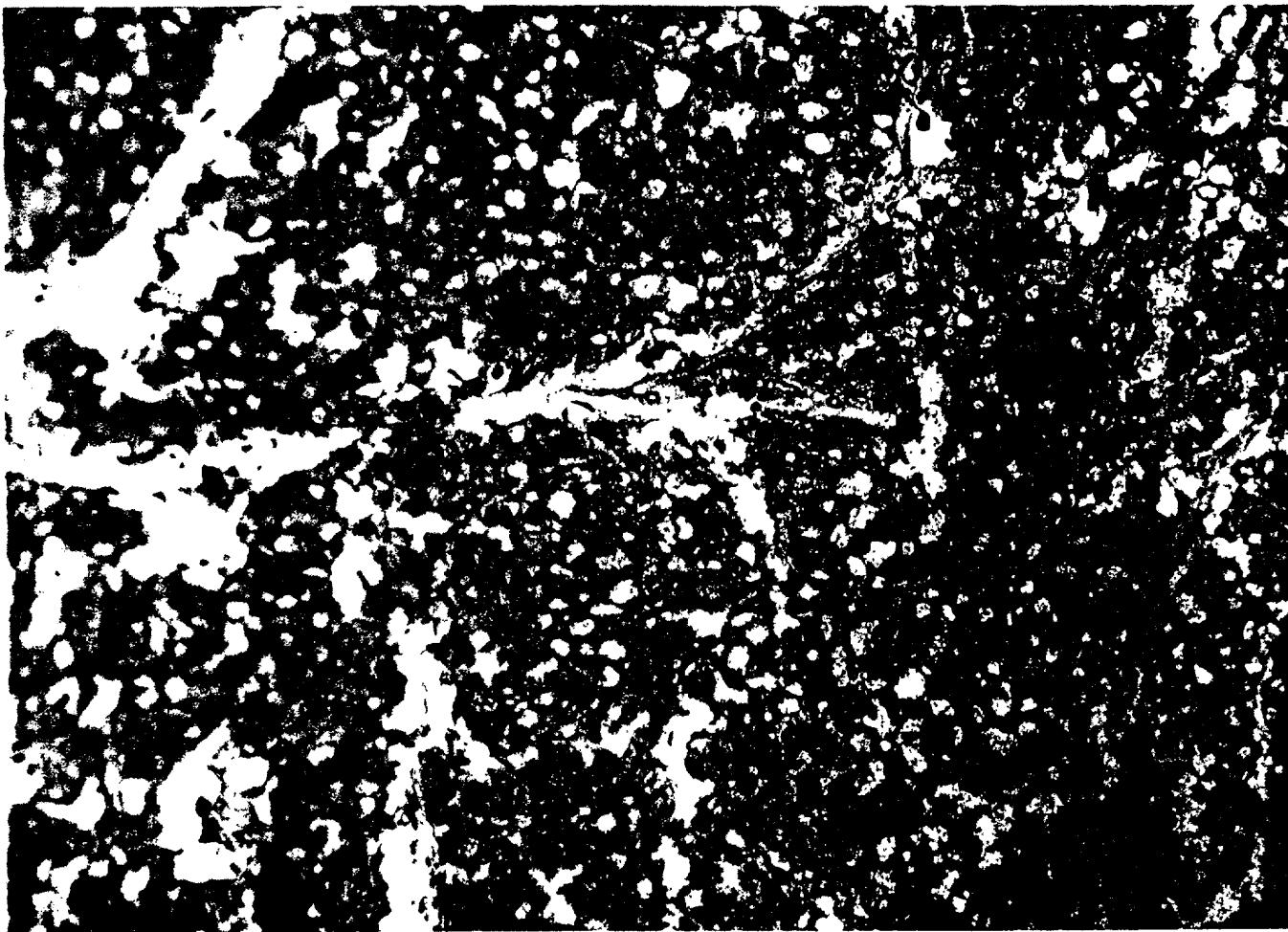


FIGURE 31

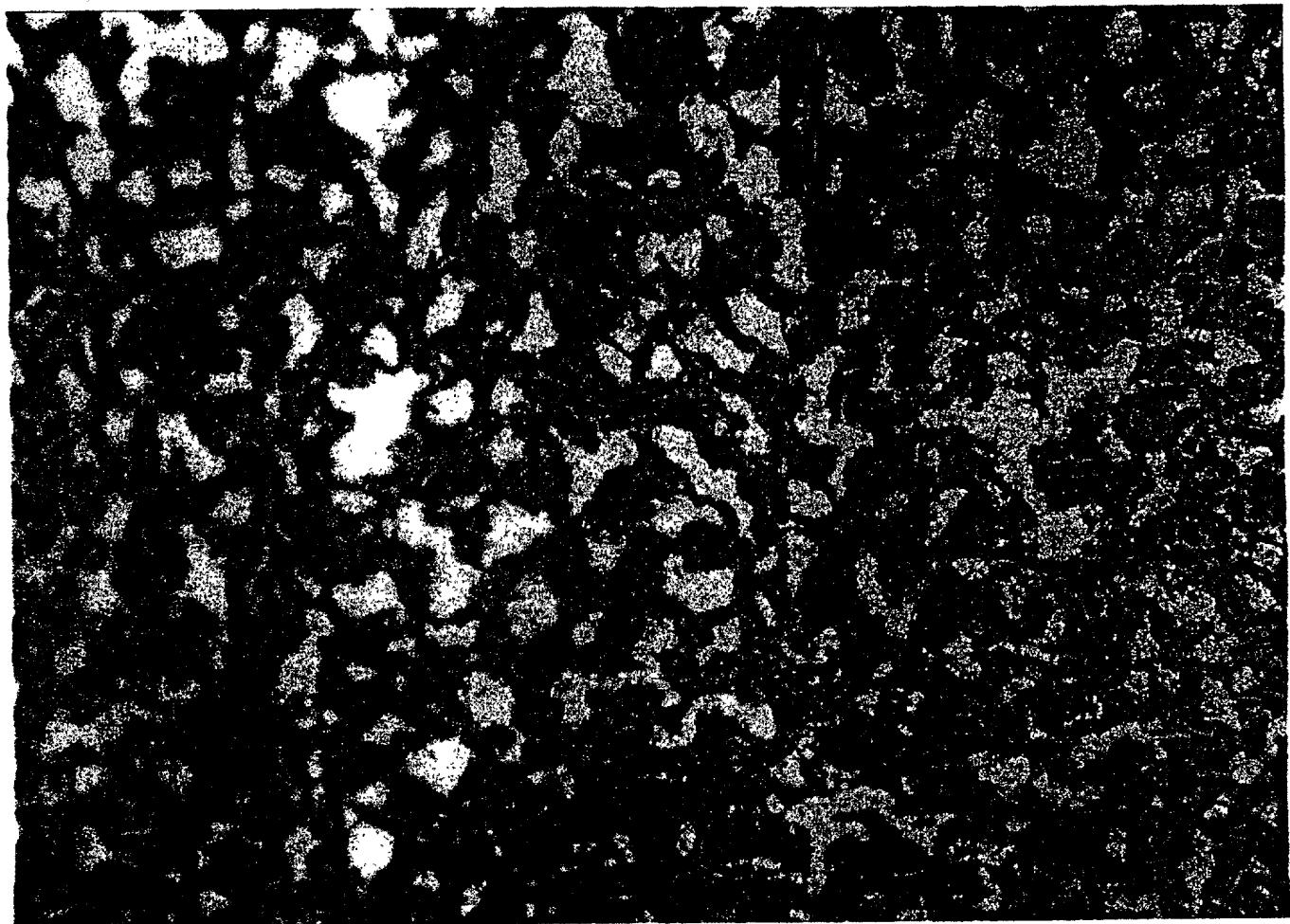


FIGURE 32

PSCA Expression in LAPC-9 Xenograft by FACS

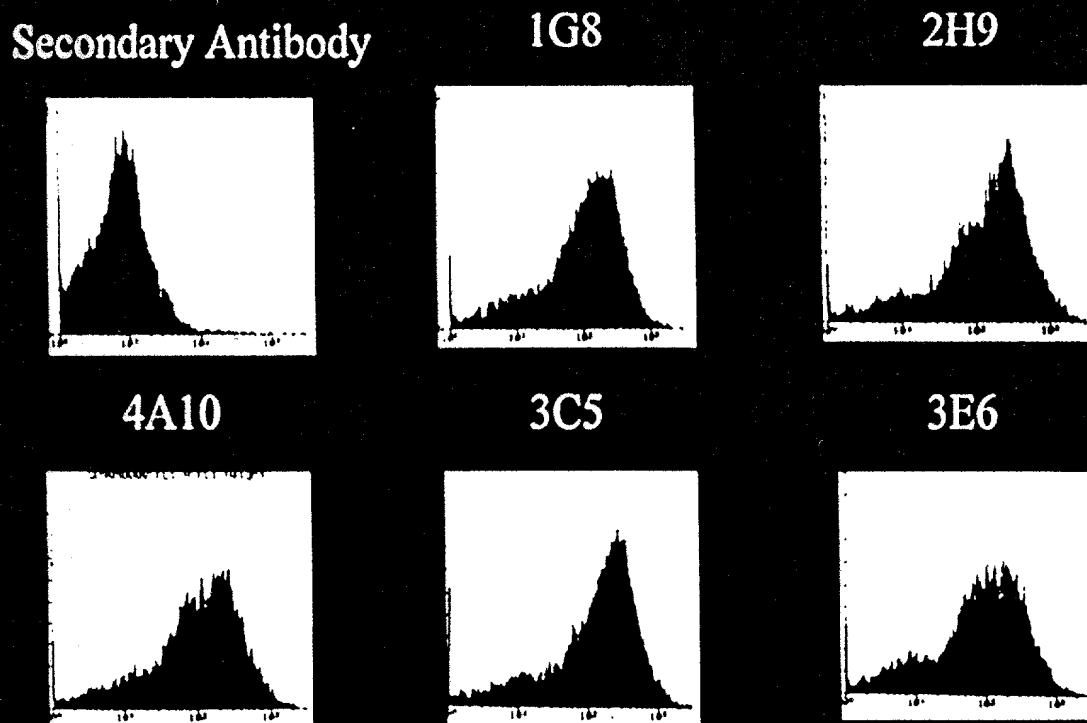


FIGURE 33

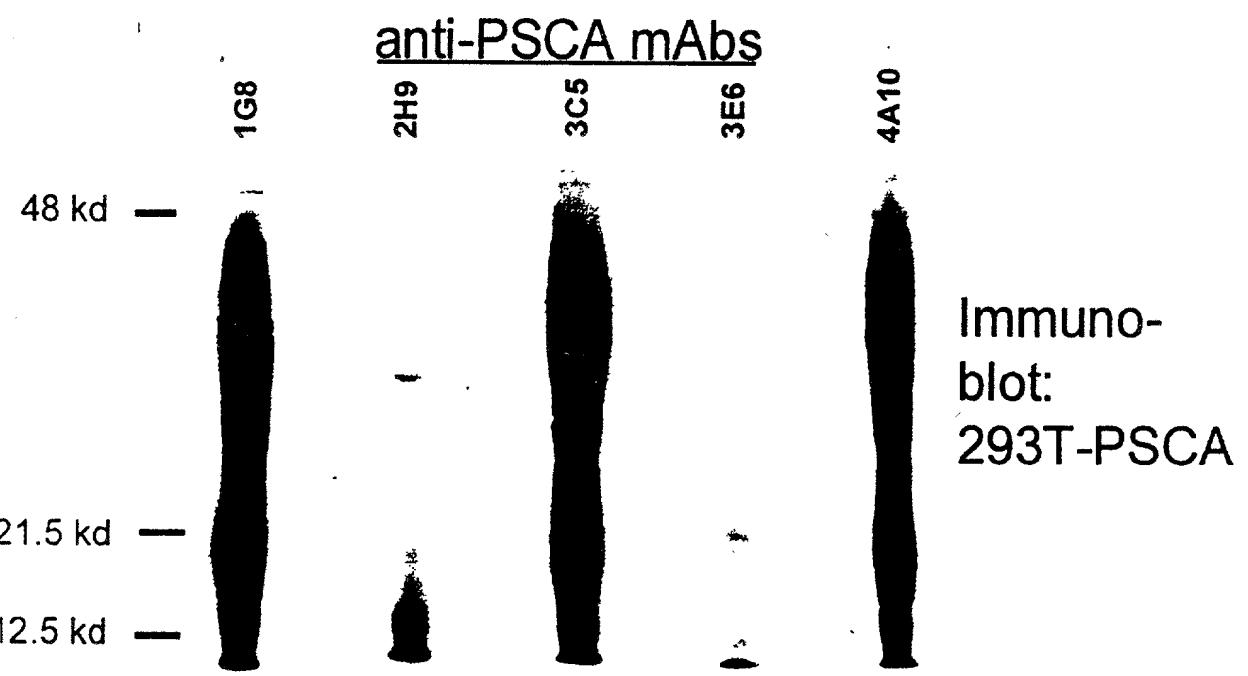


FIGURE 34

Immunofluorescent Staining of LNCaP-PSCA Cells

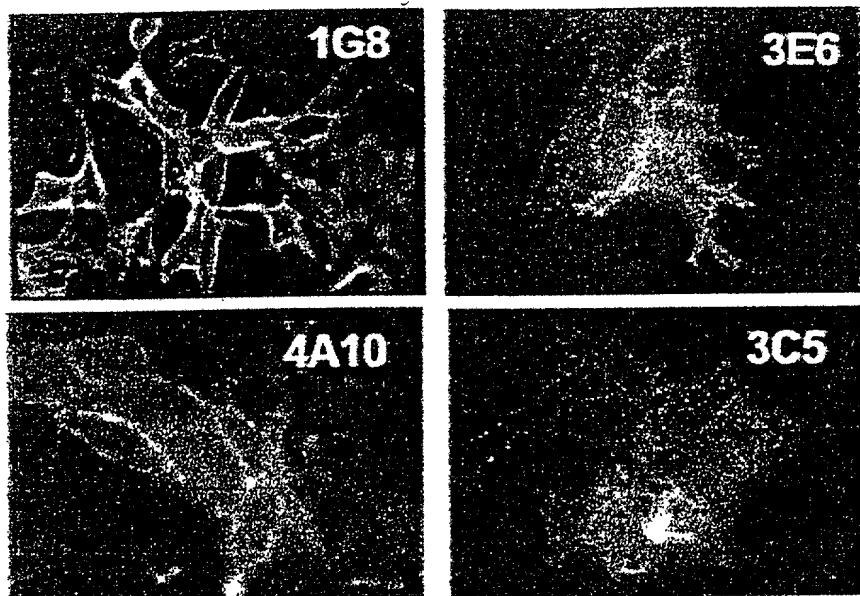


FIGURE 35

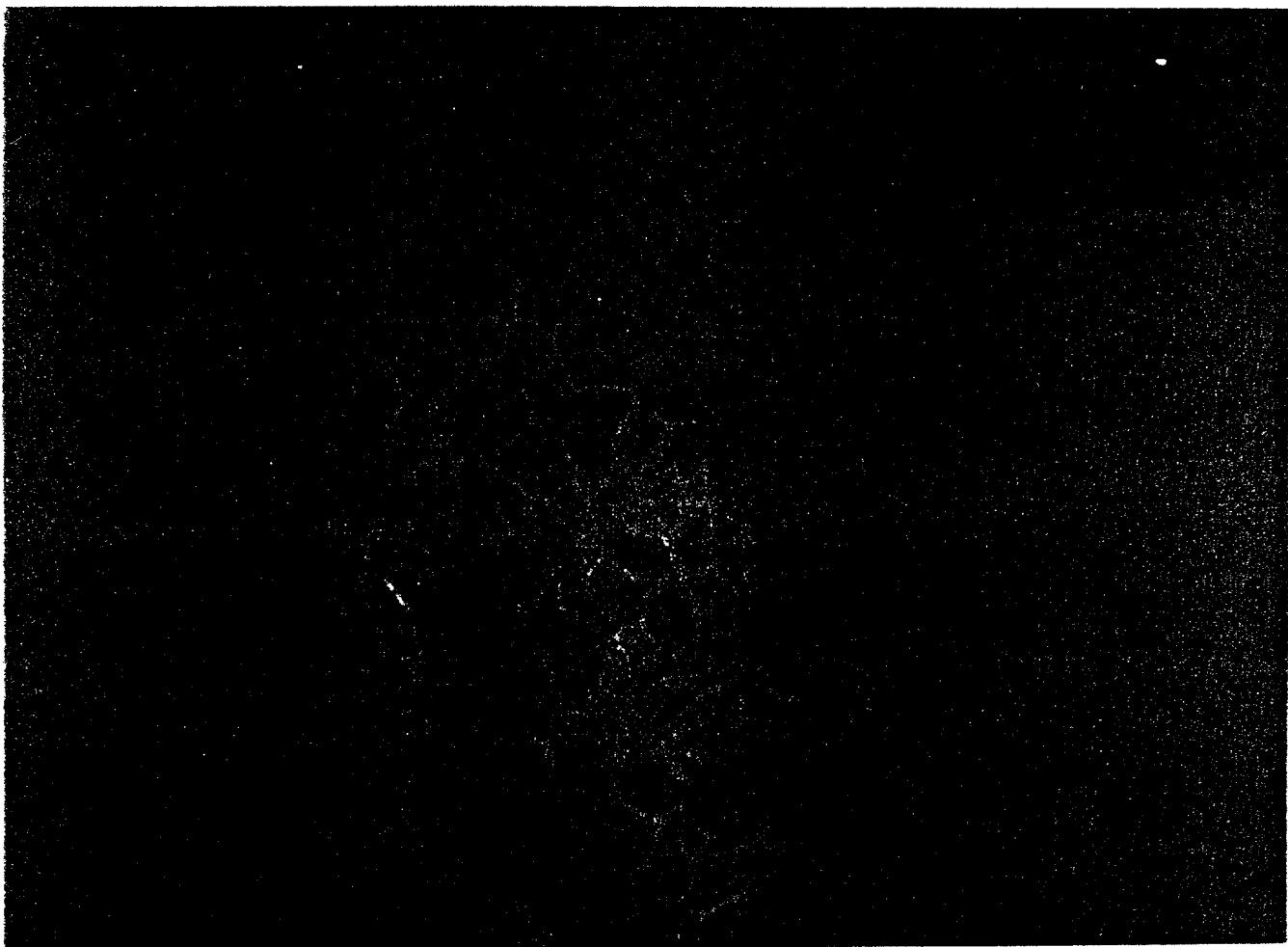


FIGURE 36

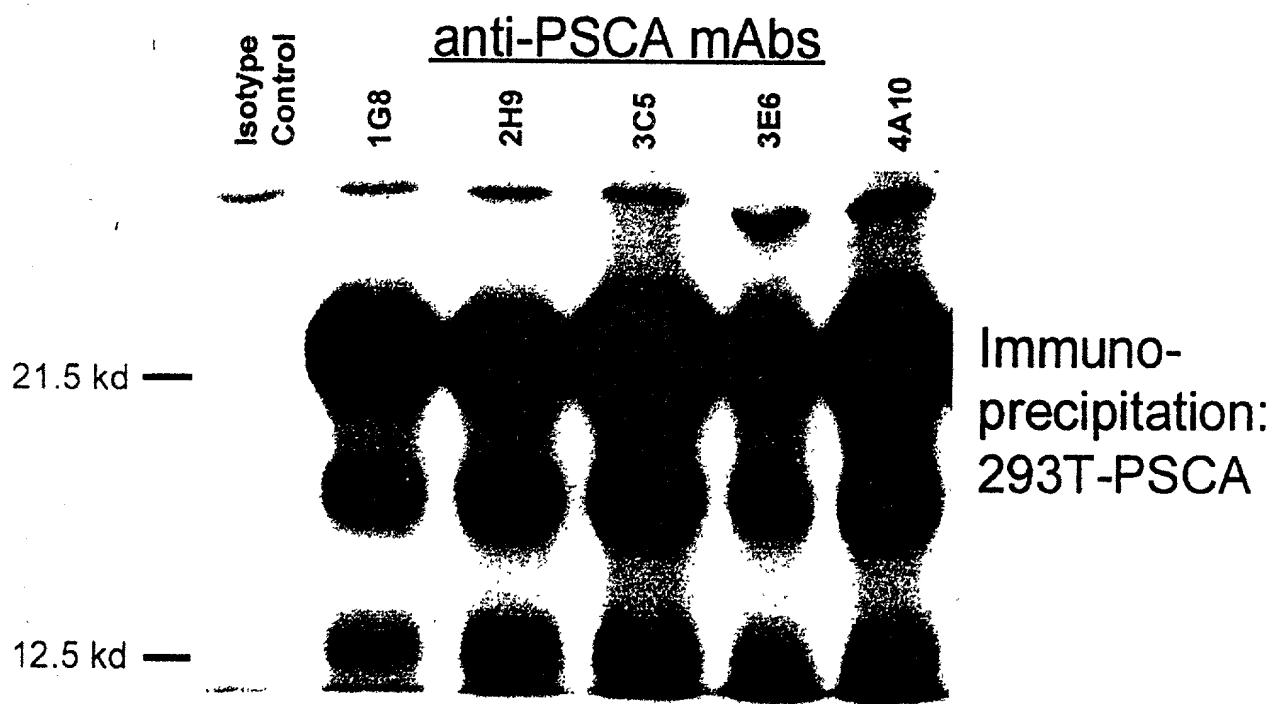
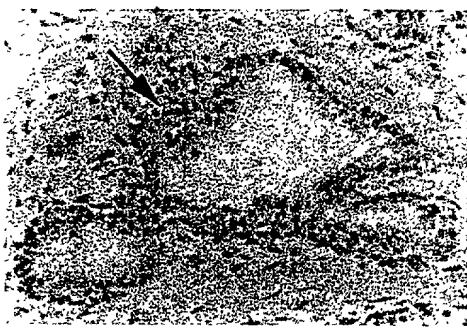


FIGURE 37

Immunohistochemical Staining of Normal Prostate

Normal: Isotype Control



Normal: PSCA mAb 3E6



Normal: PSCA mAb 1G8



Atrophy: PSCA mAb 2H9



FIGURE 38

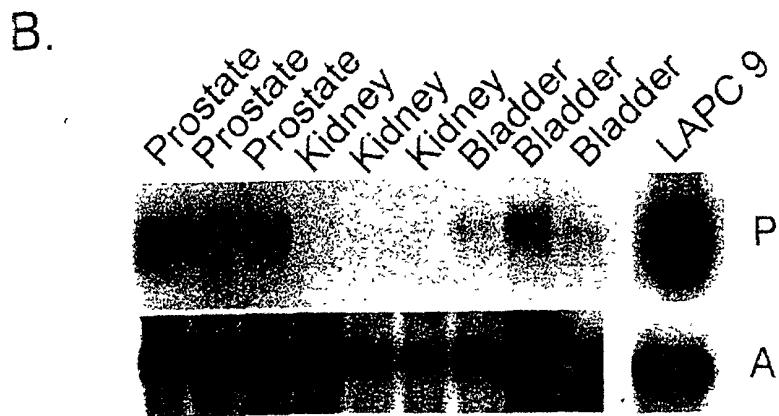
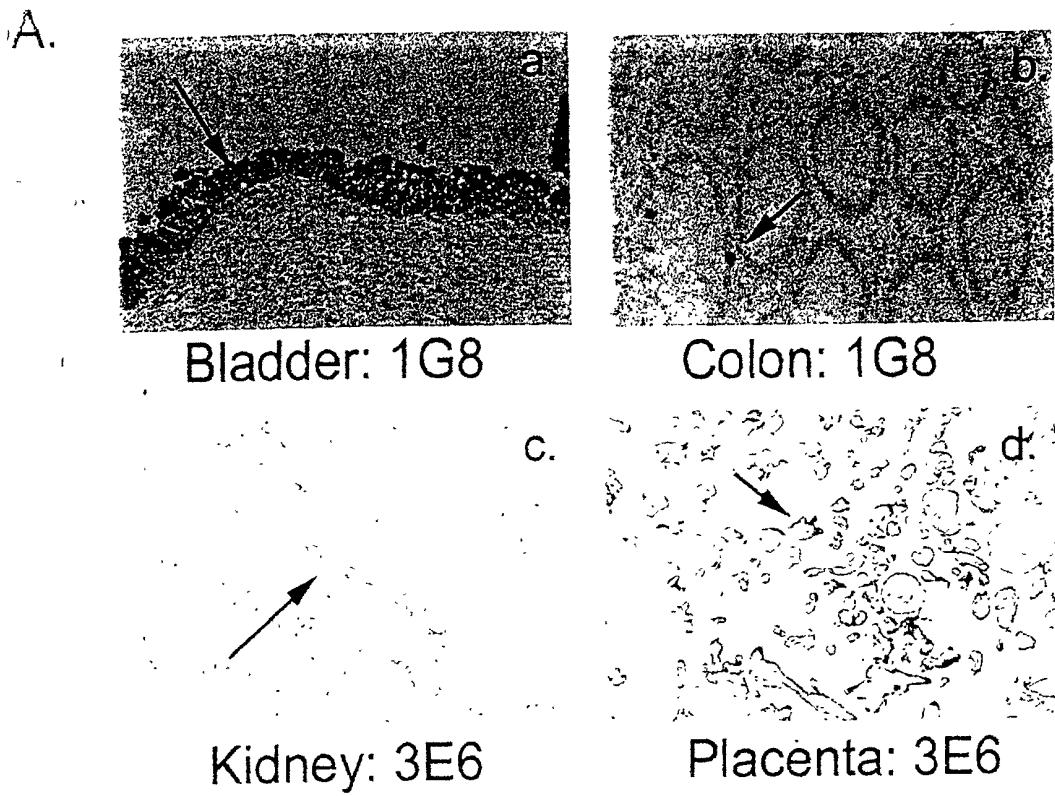
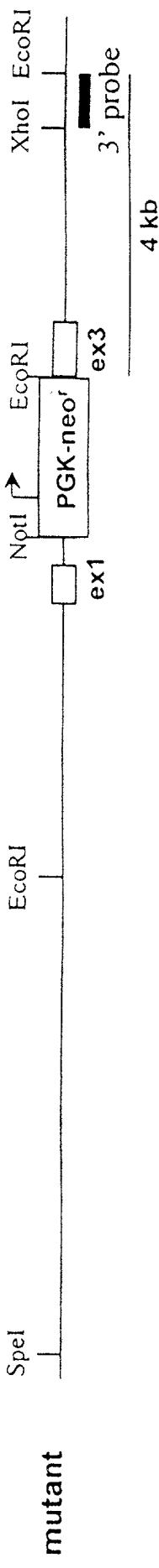
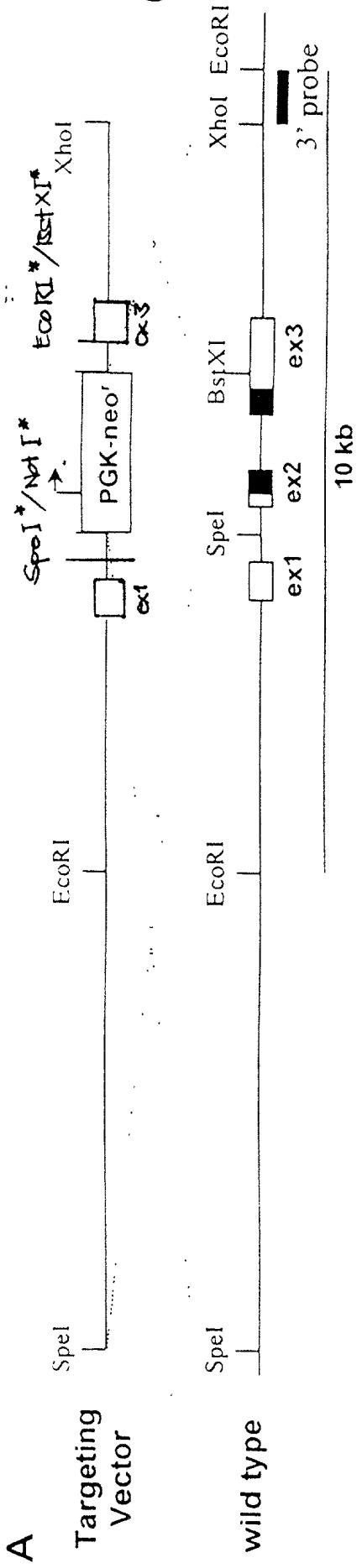


FIGURE 39

Targeting of Mouse PSCA Gene



B. Genomic Southern Analysis of ES Cells

- * ex1, 2, and 3 are the exons of PSCA gene.
- * Black boxes of ex2 and ex3 encode PSCA mature protein sequences.
- * ES genomic DNAs were digested with EcoRI, followed by Southern hybridization using 3' probe.

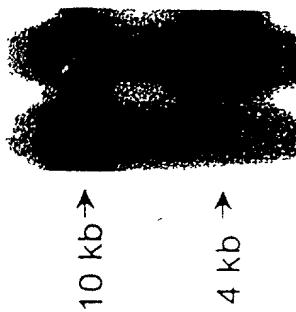
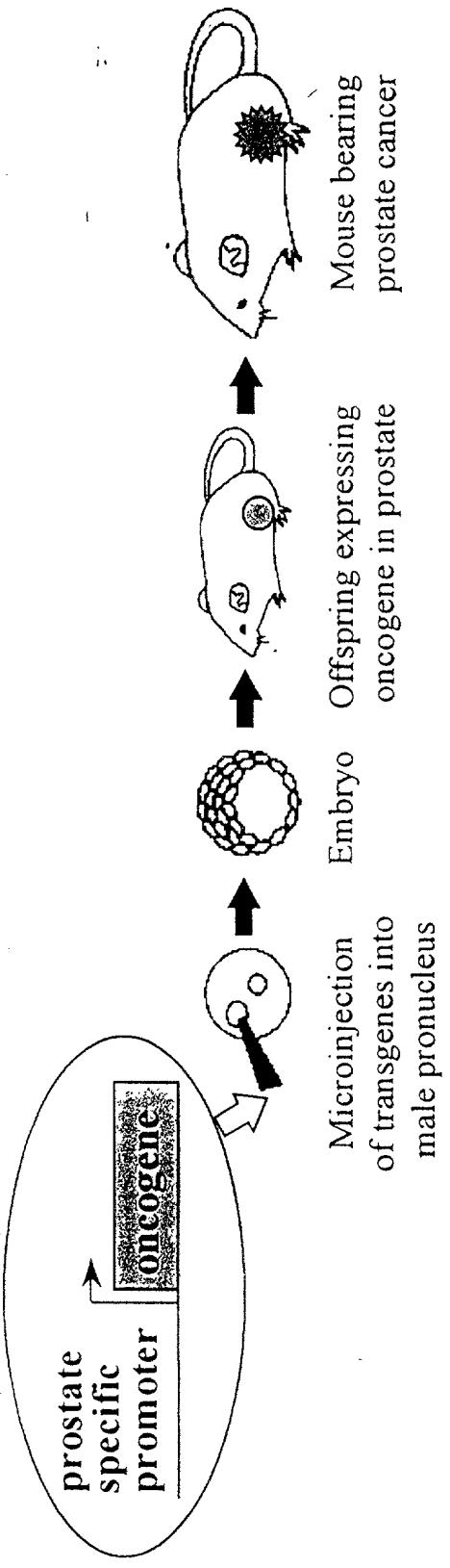


FIGURE 4C

Transgenic Mouse Models of Prostate Cancer



Transgene	Target tissues	Characteristics
C3(1) (-3 kb)/ SV40 large+small T <i>Maroulakou et al.</i> 1994 PNAS	prostate (secretory cells) urethral, mammary and sweat gland	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcinoma 28 wks No metastases
Probasin (-426 bp)/ SV40 large+small T <i>Greenberg et al.</i> 1995 PNAS	prostate (secretory cells)	Low-grade PIN 5-8 wks High-grade PIN 8-12 wks Invasive carcinoma 12 wks Metastases in lymph node, lung, liver and bone
Cryptdin2 (-6.5 kb)/ SV40 large+small T <i>Garabedian et al.</i> 1998 PNAS	prostate (neuroendocrine cells) small intestine	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcinoma 16 wks Metastases in lymph node, lung, liver and bone

FIGURE 41

Reporter Gene Constructs for Transfection Assay

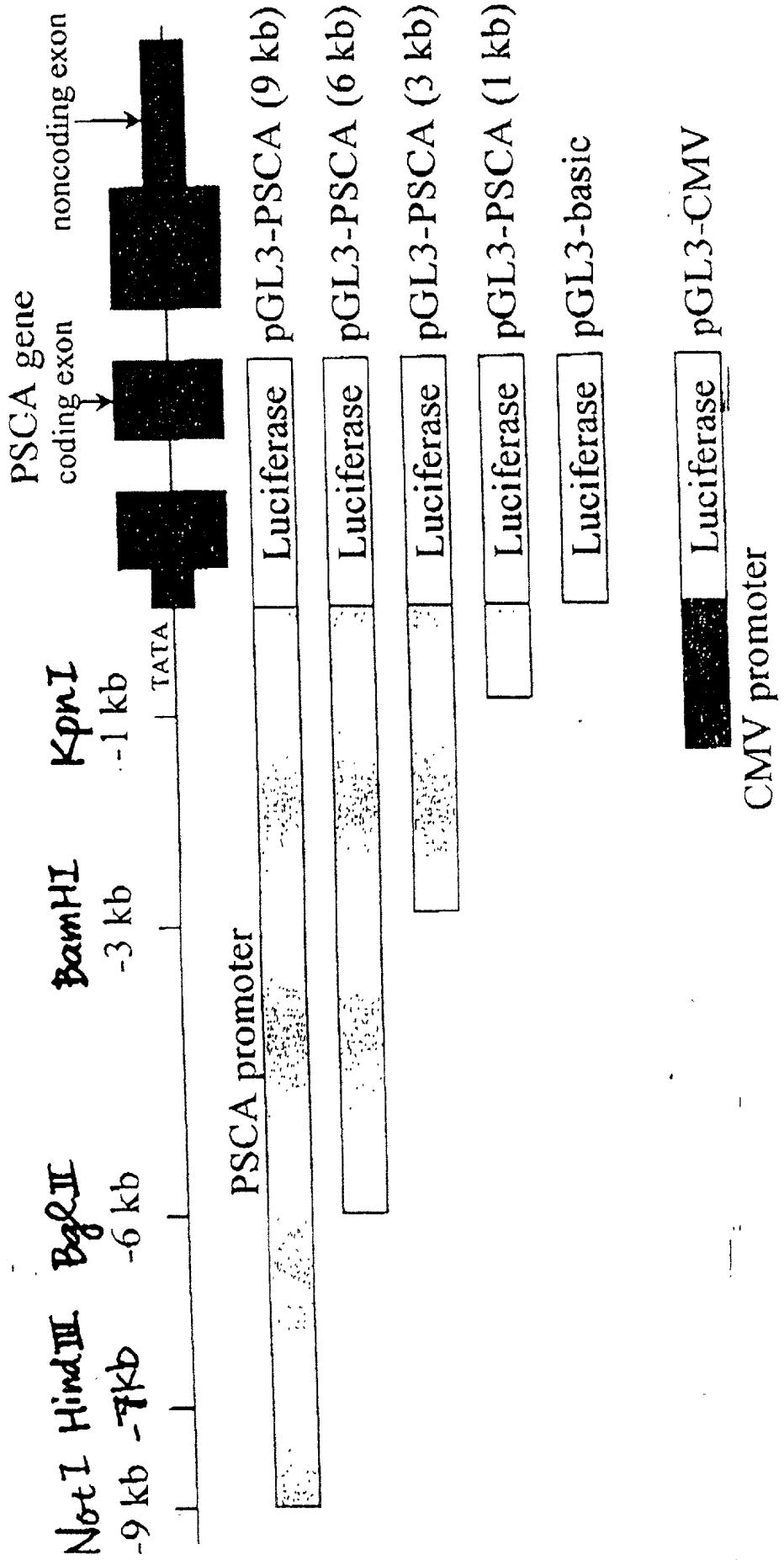
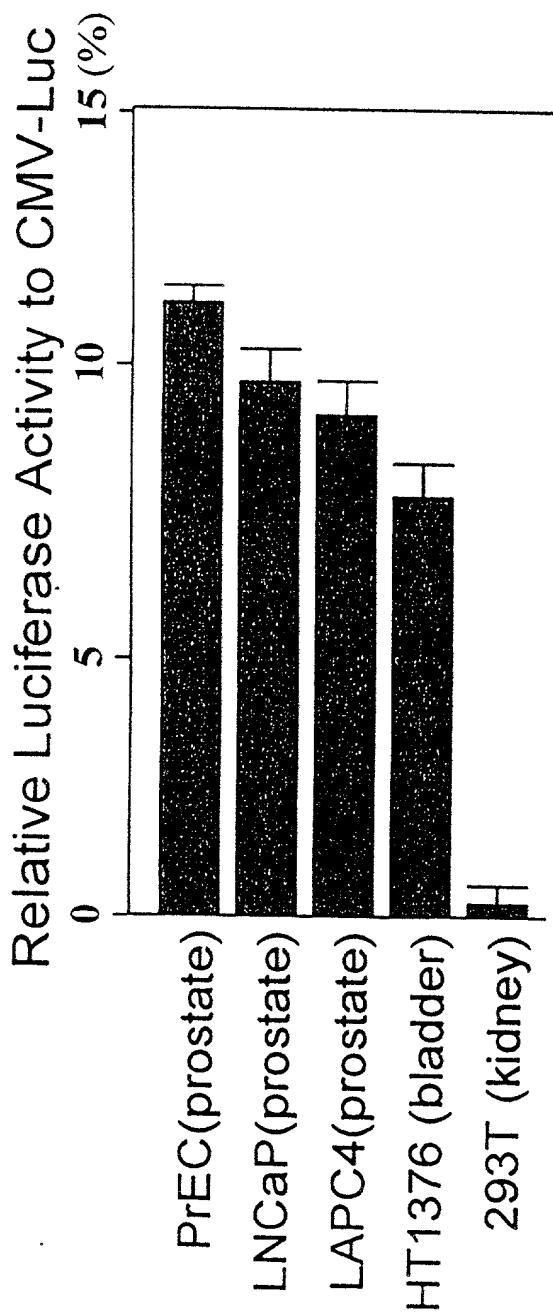


FIGURE 42

FIGURE 43



Identification of Prostate-Specific Elements Within PSCA Promoter Sequences

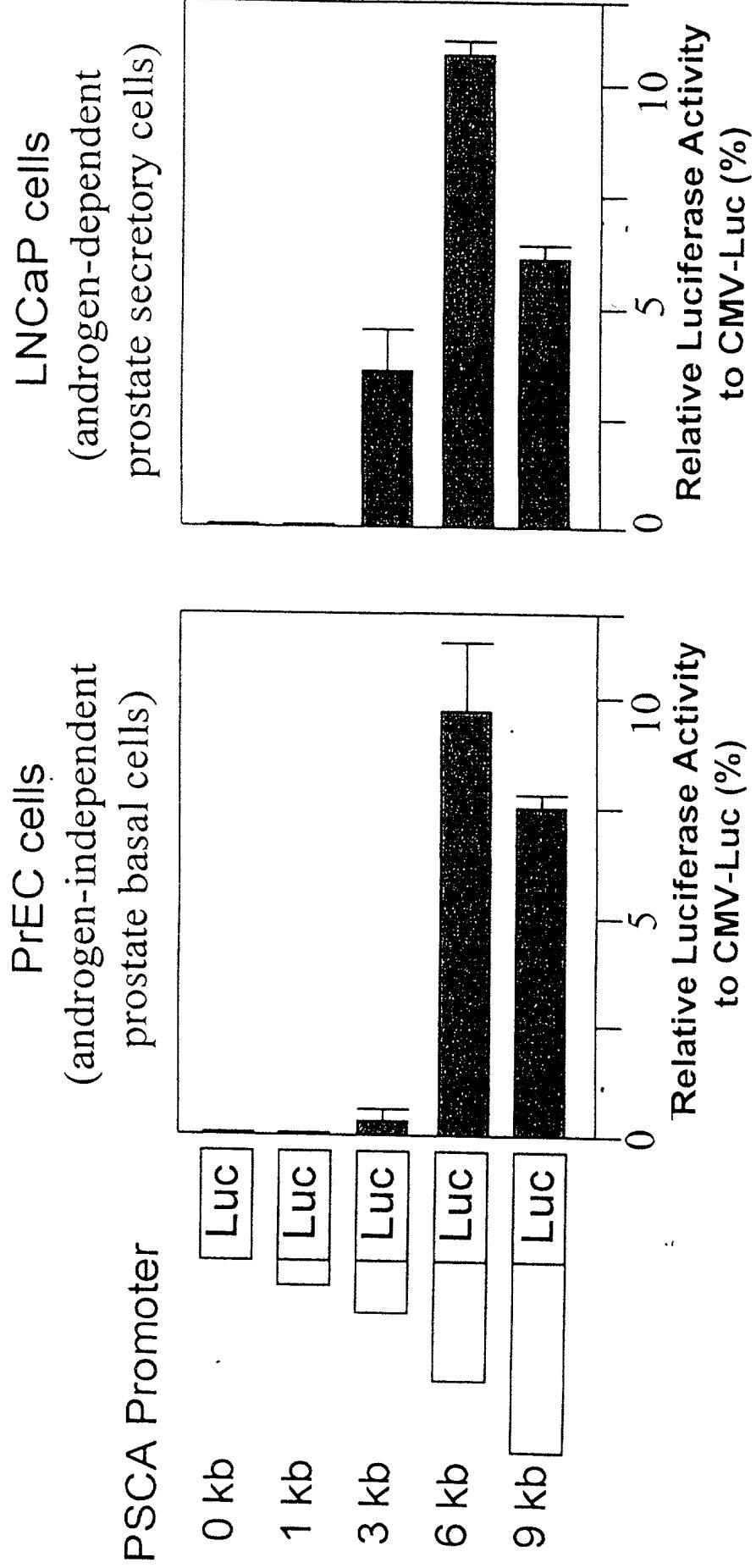


FIGURE 44

Update of Transgenic Mouse Projects

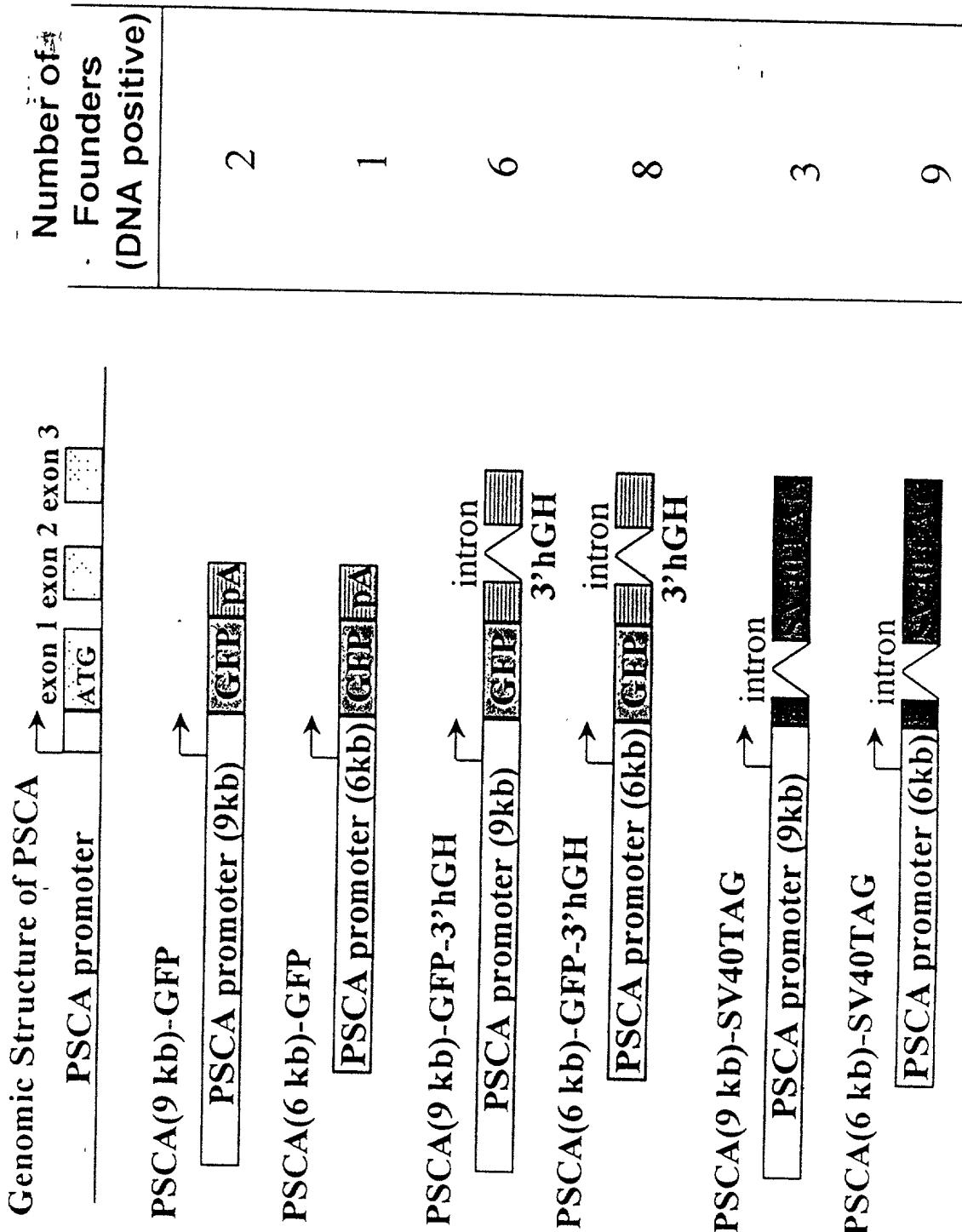


FIGURE 45

<u>Negative tissues</u>	
Stomach	
Small intestine	
Colon	
Seminal Vesicle	
Urethra	
Testis	
Liver	
Kidney	
Lung	
Brain	
Heart	
Skeletal muscle	
Ovary	
Uterus	

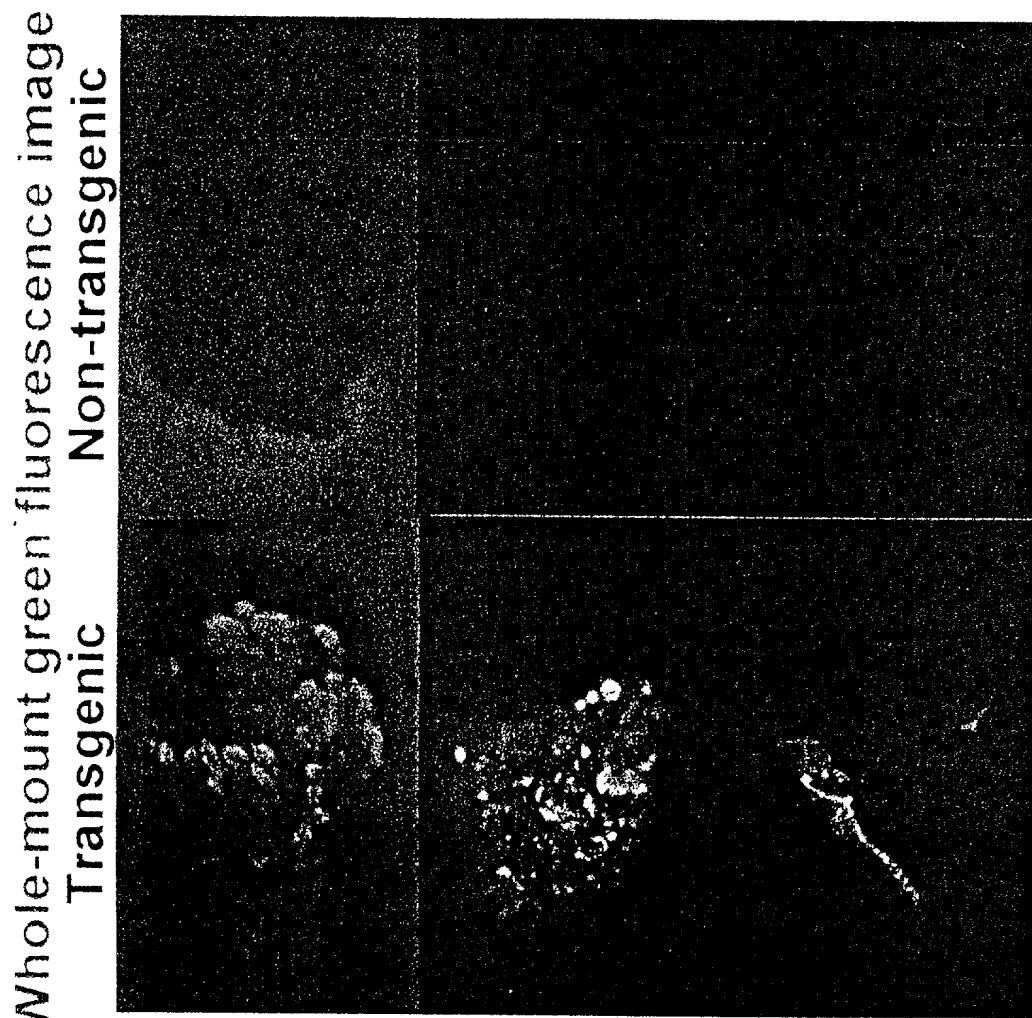


FIGURE 47

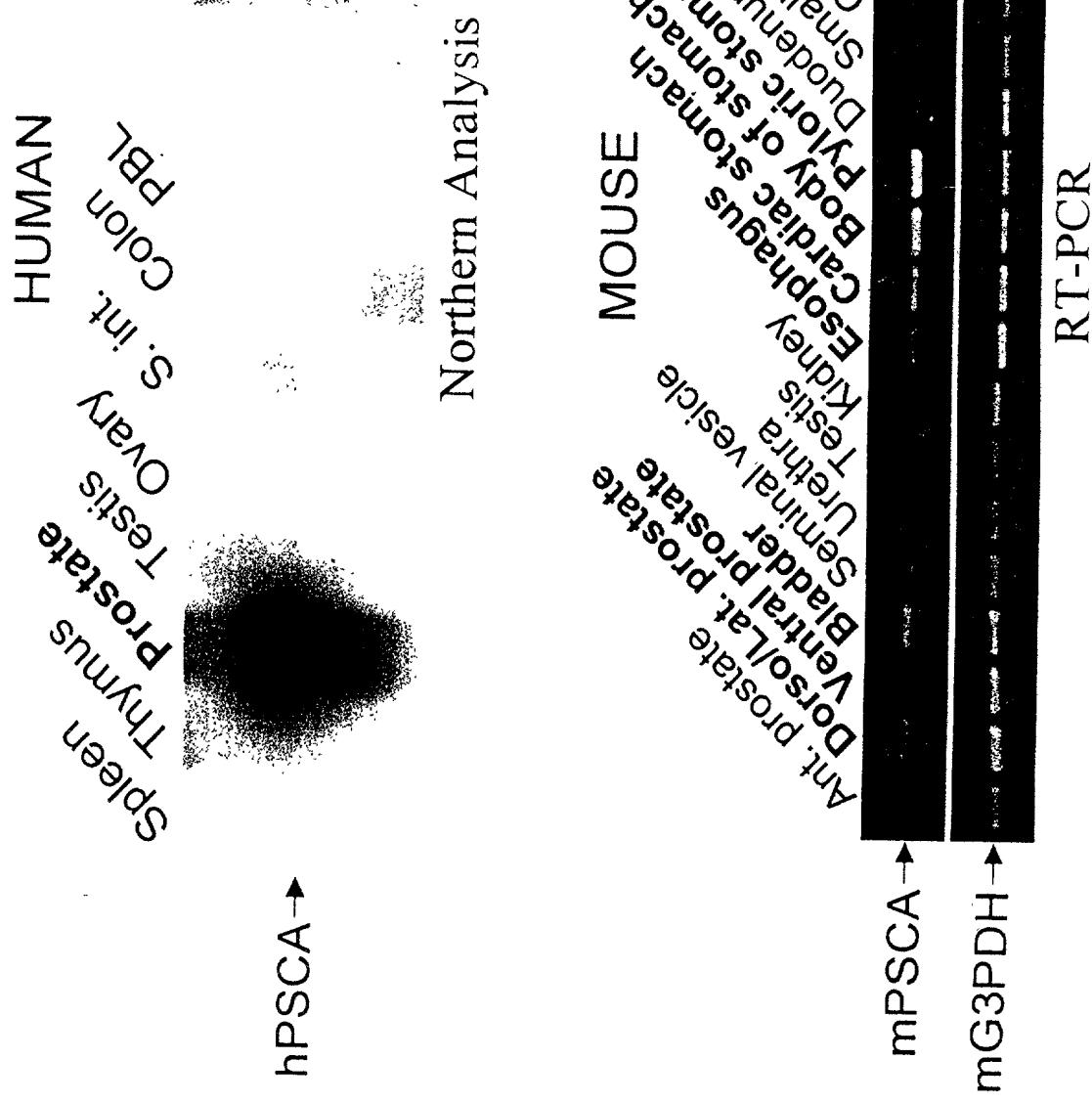
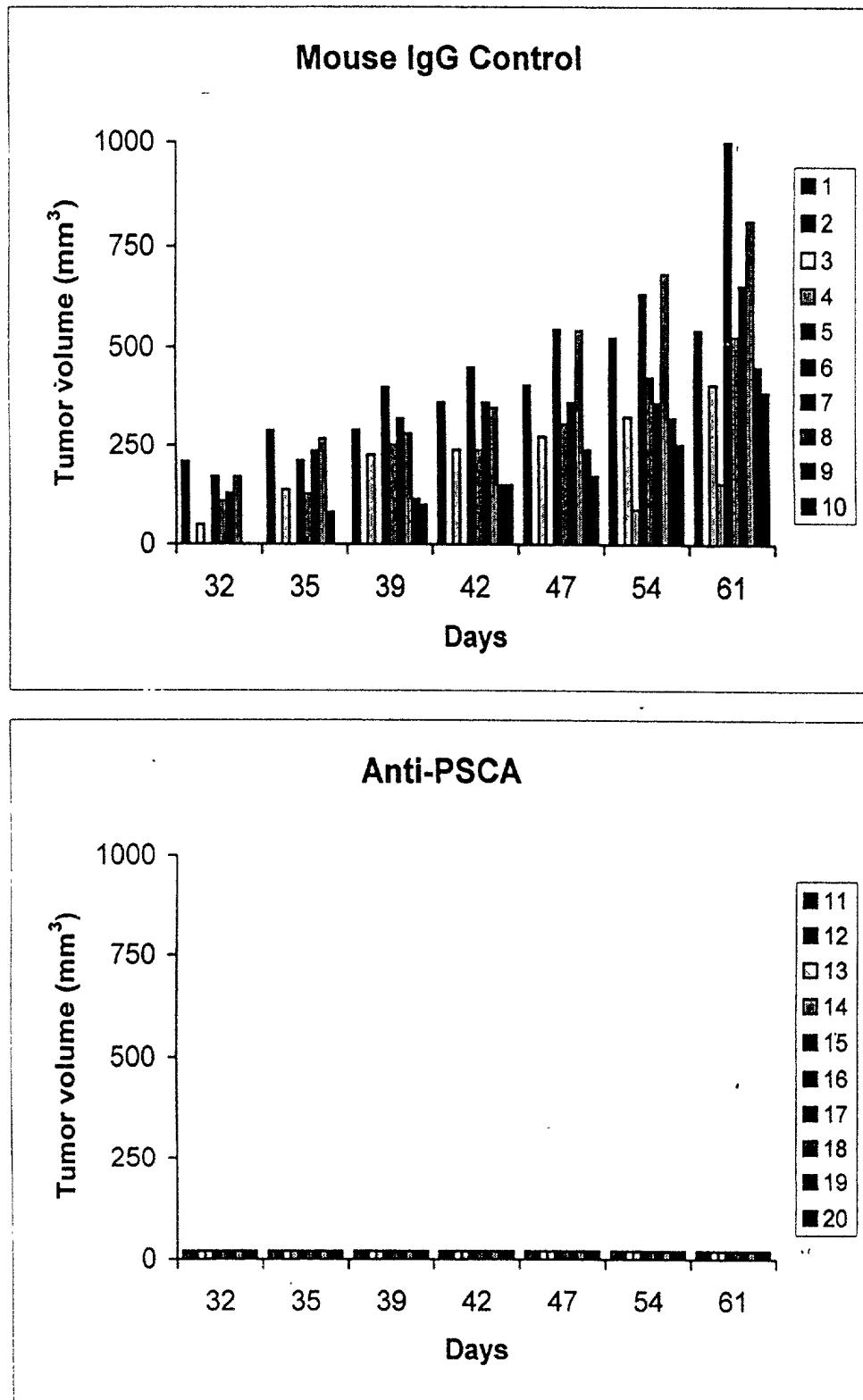


FIG. 48



A

Epitope recognized (OD 450 nm)

<u>mAb</u>	<u>Isotype</u>	<u>F(18-98)</u>	<u>N(2-50)</u>	<u>M(46-109)</u>	<u>C(85-123)</u>
1G8	IgG1 K	1.485	0.004	1.273	0.003
2A2	IgG2a K	0.973	0.631	0.023	0.010
2H9	IgG1 K	1.069	1.026	0.002	0.001
3C5	IgG2a K	1.916	1.709	0.006	0.002
3E6	IgG3 K	1.609	0.036	1.133	2.118
3G3	IgG2a K	2.805	1.731	0.004	0.000
4A10	IgG2a K	1.053	0.493	0.000	0.001

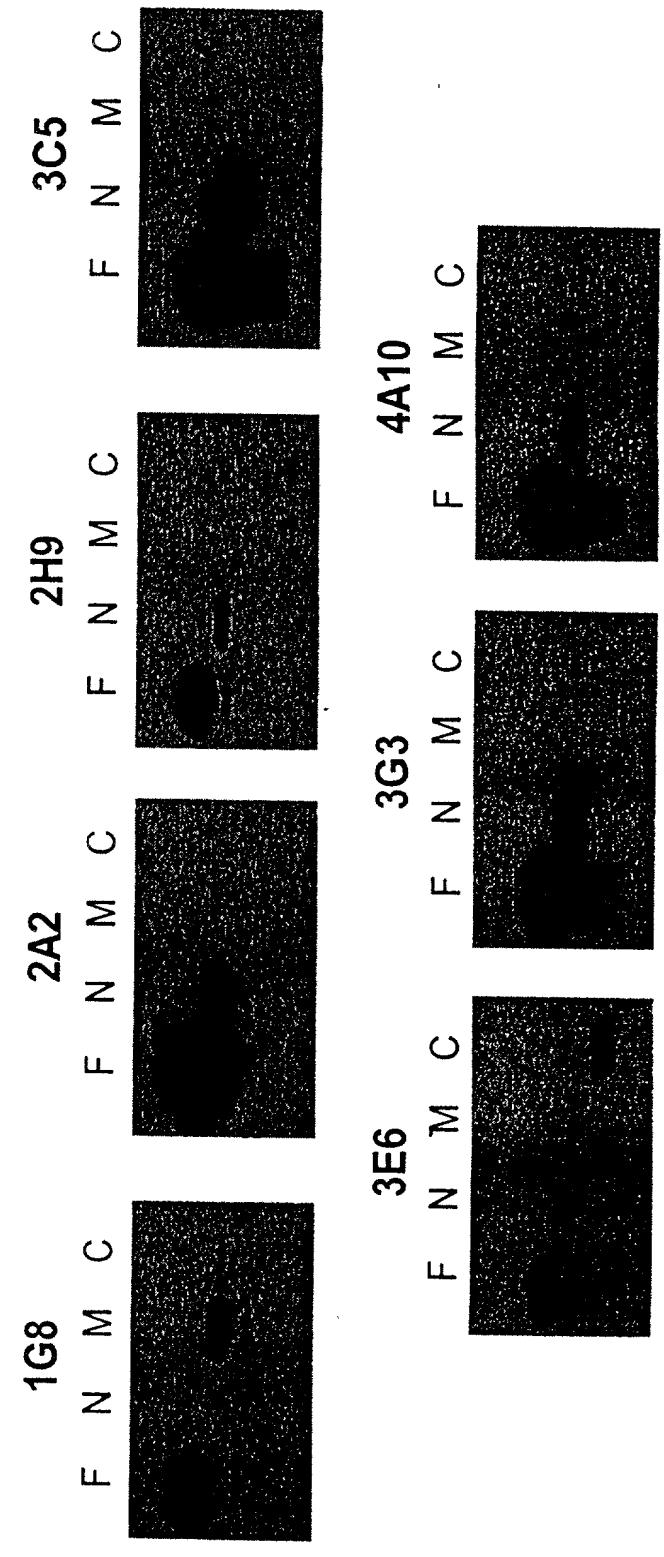
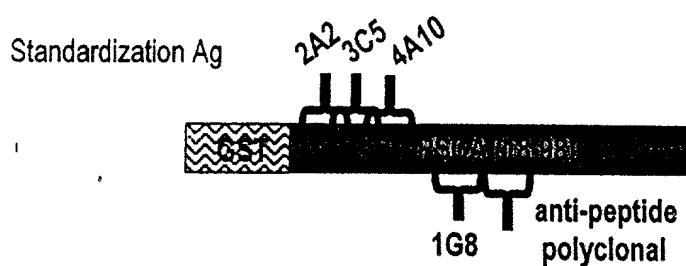
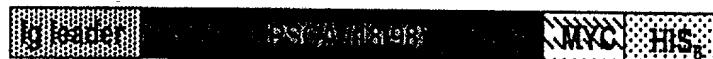
FIG. 49

FIG. 50

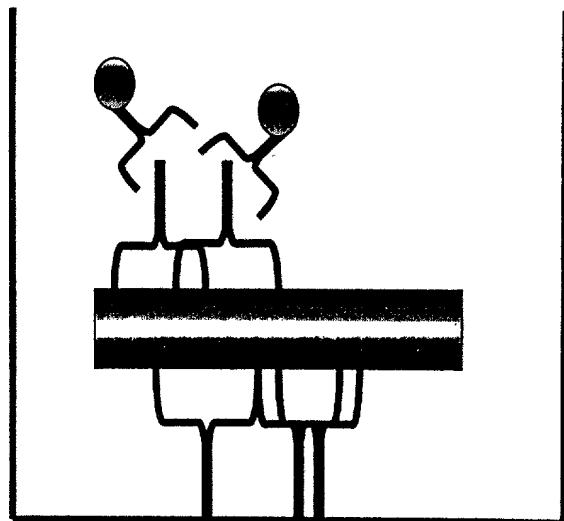
A



Engineered mammalian secreted form



B



Anti-IgG2a HRP

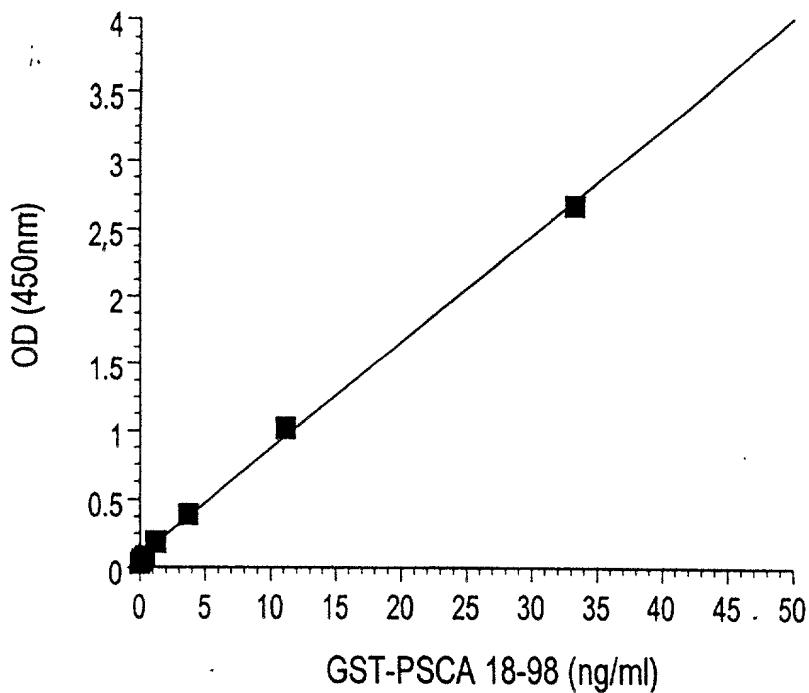
Anti-PSCA mAbs 3C5+4A10+2A2 (IgG2a)

PSCA

Affinity purified anti-peptide polyclonal
+ mAb 1G8 (IgG1)

FIG. 51

A



B

<u>Sample</u>	<u>OD+range (n=2)</u>	<u>ng/ml</u>
vector	0.005+0.001	ND
vector+hu serum	0.004+0.001	ND
secPSCA	2.695+0.031	32.92
secPSCA+hu serum	2.187+0.029	26.55

FIG. 52

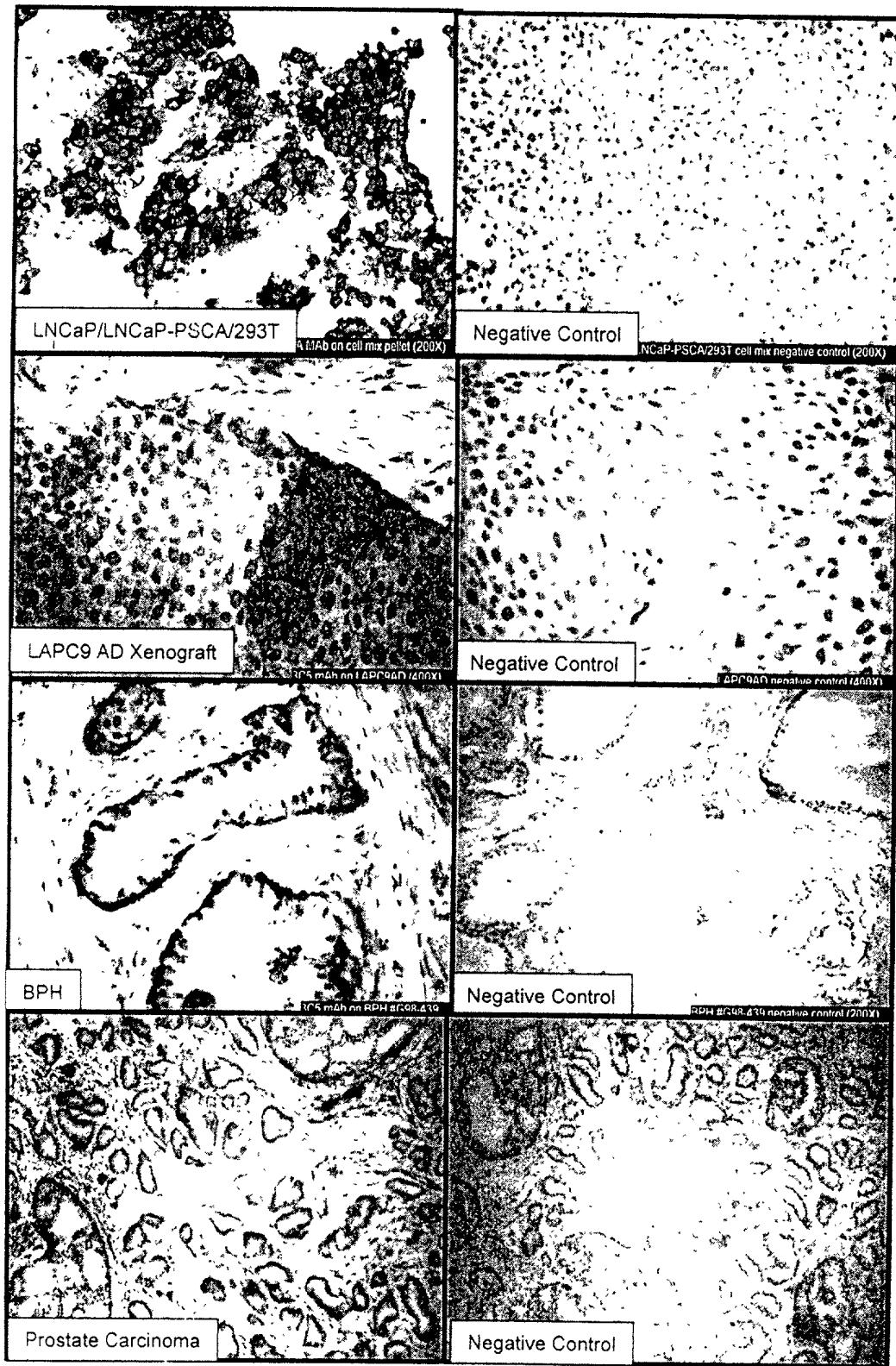


FIG. 53

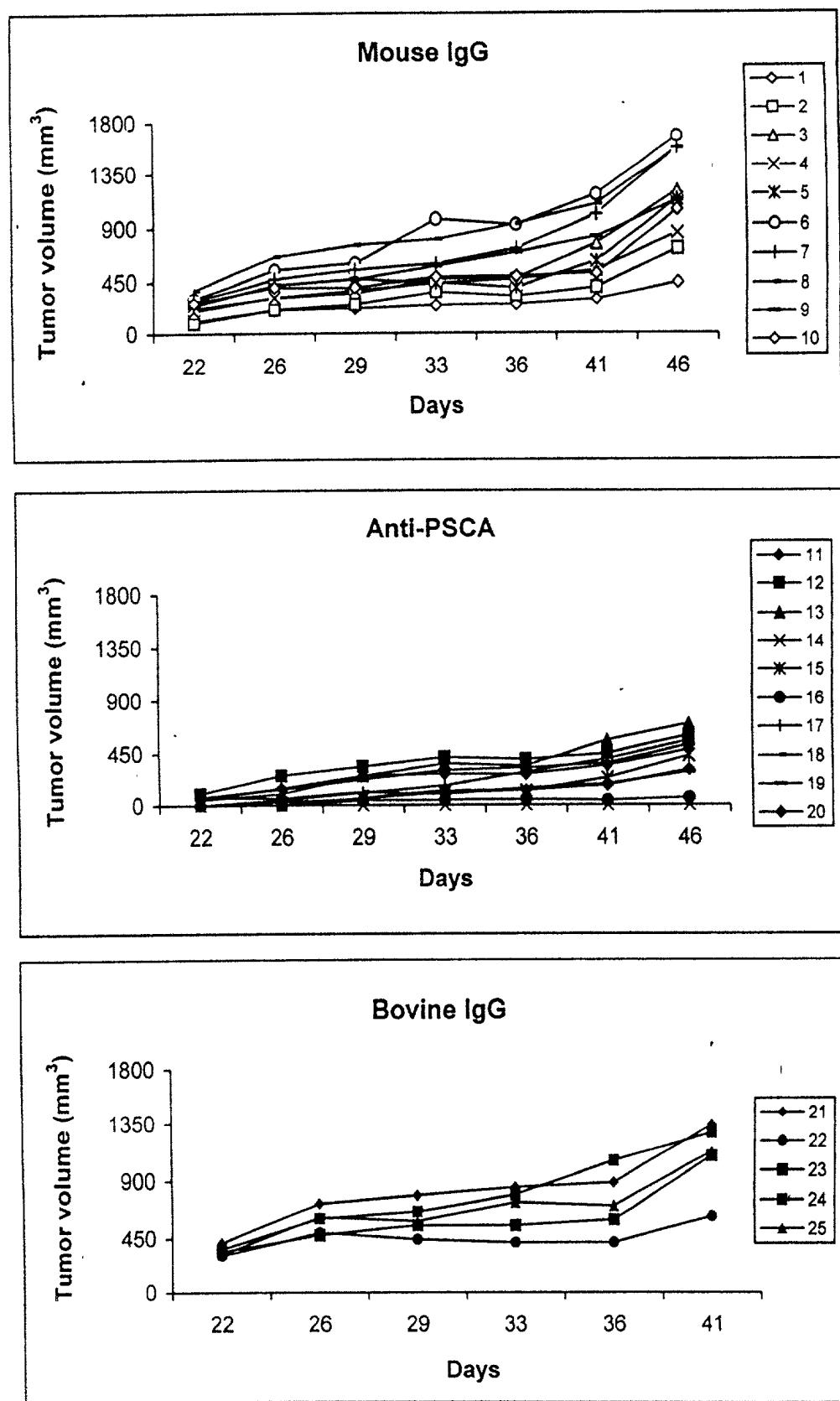


FIG. 54

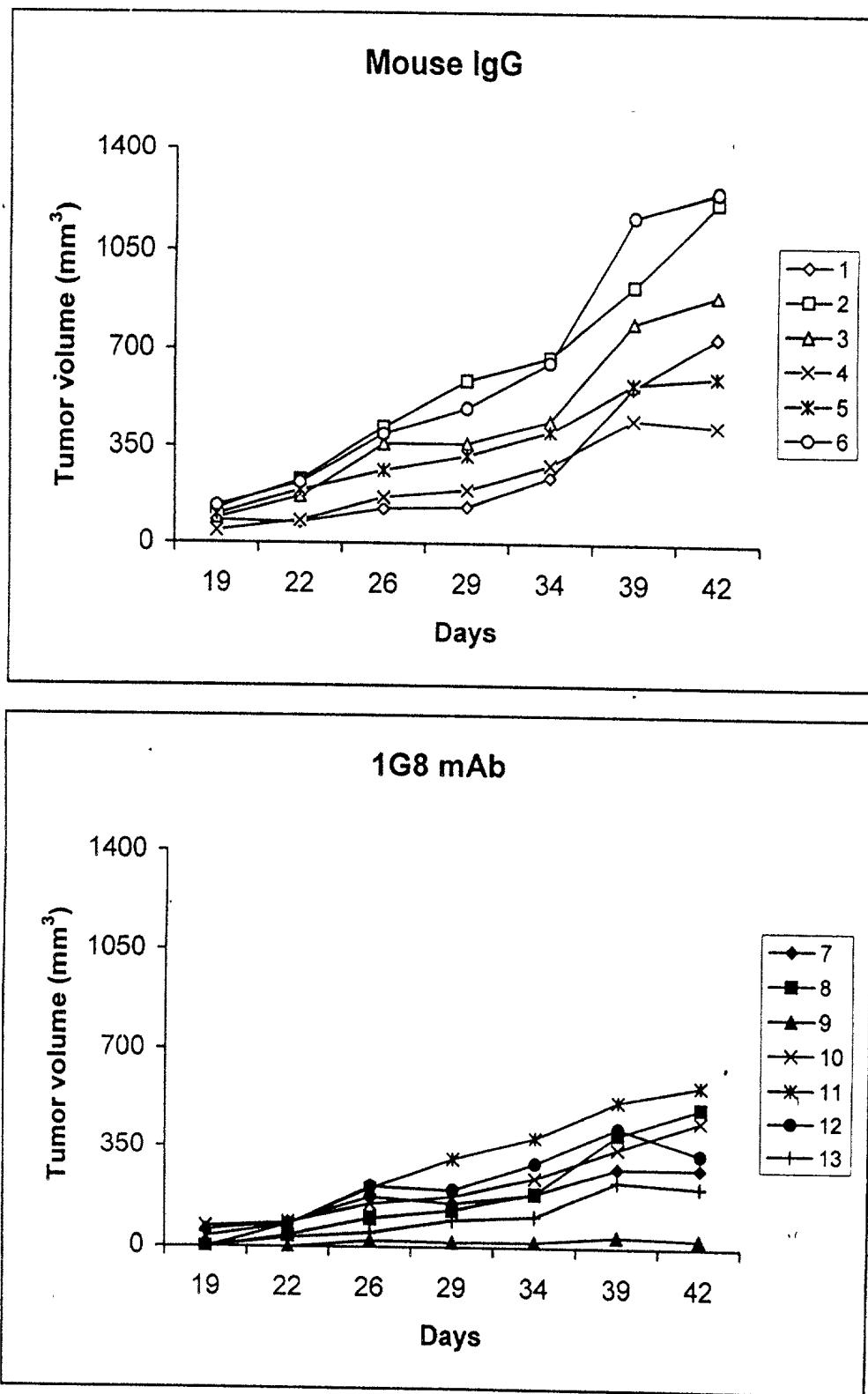


FIG. 55

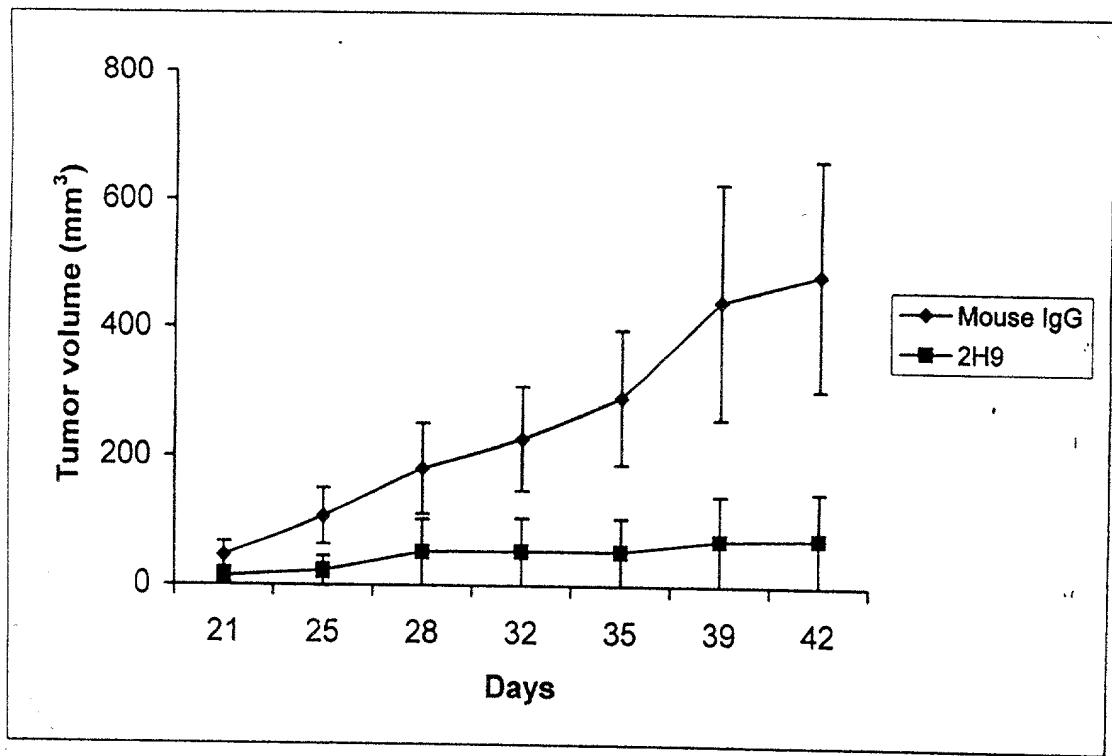
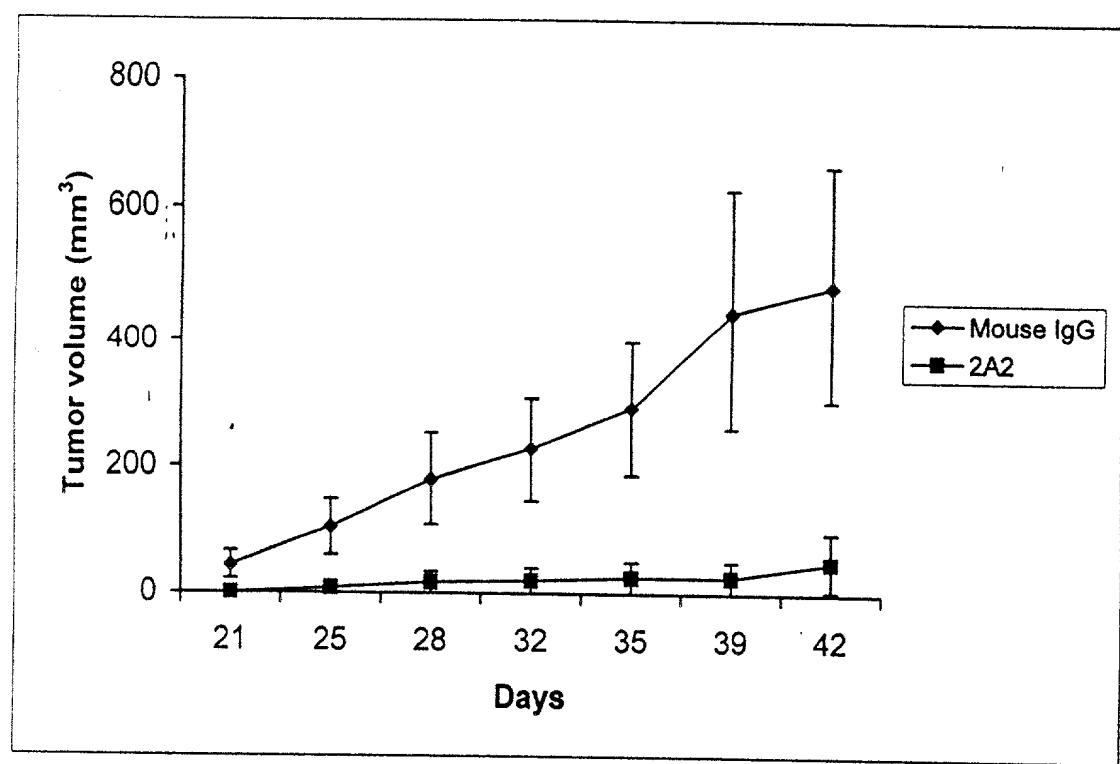


FIG. 56

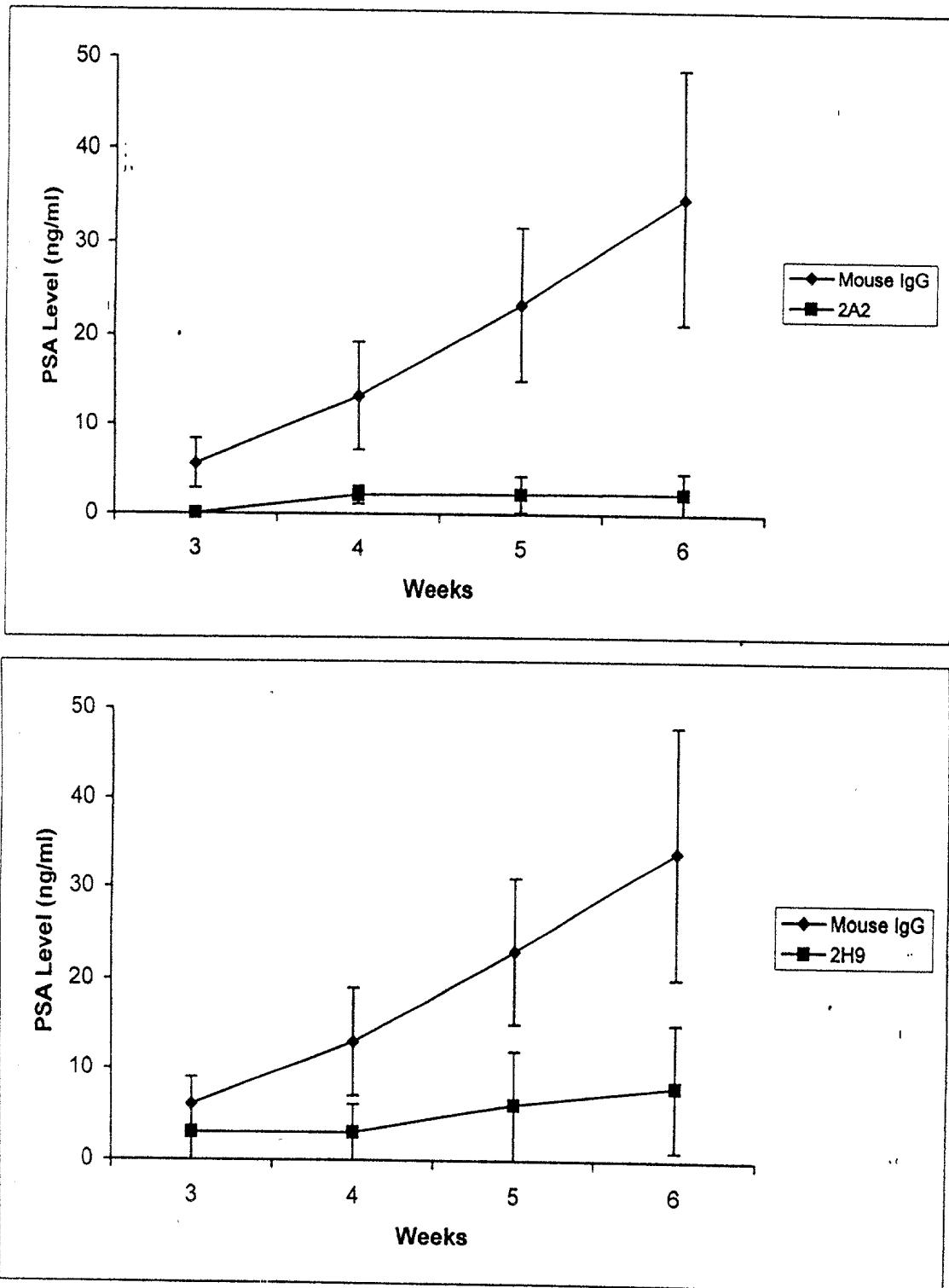


FIG. 57

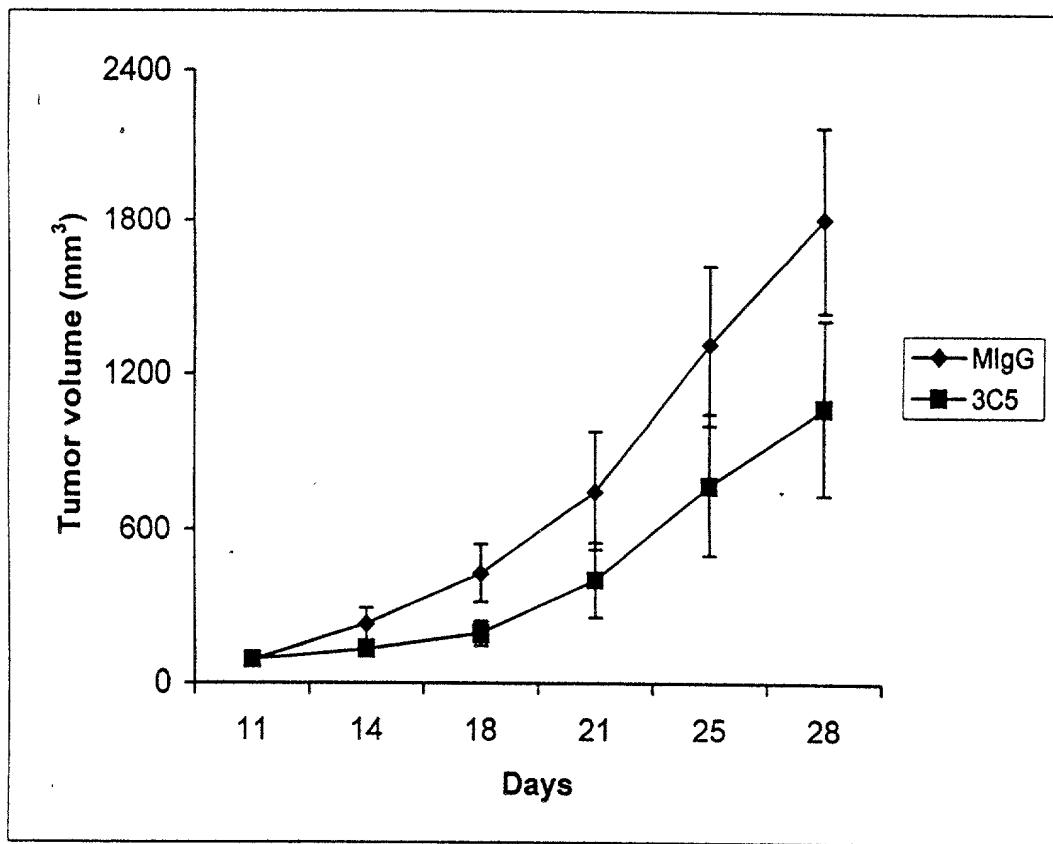


FIG. 58

TGCTTCTCCTGATGGCAGTGGTTAGGAGTCATTAGGAGTCAGAGGTTCAAGCTGCAGCAGTCT 60
C F F L M A V V I G V N S E V Q L Q Q S 20
.
GGGGCAGAACTTGTGAGGTCAGGGCCTCAGTCAGTTGTCCGCACAGCTCTGGCTTC 120
G A E L V R S G A S V K L S C T A S G F 40

CDR1
AACATTAAAGACTACTATACACTGGGTGAATCAGAGGCCTGACCAGGGCCTGGAGTGG 180
N I K D Y Y I H W V N Q R P D Q G L E W 60

CDR2
ATTGGATGGATTGATCCTGAGAATGGTGACACTGAATTGTCCCGAAGTTCCAGGGCAAG 240
I G W I D P E N G D T E F V P K F Q G K 80

GCCACTATGACTGCAGACATTTCTCAAACACAGCCTACCTGCACCTCAGCAGCCTGACA 300
A T M T A D I F S N T A Y L H L S S L T 100

CDR3
TCTGAAGACACTGCCGTCTATTACTGTAAAACGGGGGTTCTGGGGCCAAGGGACTCTG 360
S E D T A V Y Y C K T G G F W G Q G T L 120

GTCACTGTCTCTGCAGCCAAACGACACCCCCATCTGTCTATCCACTG
V T V S A A K T T P P S V Y P L

FIG. 59

TTGGTAGCAACAGCCTCAGATGTCCACTCCCAGGTCCAAC TGAGCAACCTGGGTCTGAA 60
L V A T A S D V H S Q V Q L Q Q P G S E 20

CTGGTGAGGCCTGGAAC TTCA GTGA AGCTGTCCTGCAAGGCTTCTGGCTATACATTCTCC 120
L V R P G T S V K L S C K A S G Y T F S 40
CDR1

AGCTACTGGATGC ACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAAAT 180
S Y W M H W V K Q R P G Q G L E W I G N 60

ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
I D P G S G Y T N Y A E N L K T K A T L 80
CDR2

ACTGTAGACACAT CCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGAC 300
T V D T S S S T A Y M Q L S S L T S E D 100

TCTGCAGTCTATTACTGTACAAGCCGATCTACTATGATTACGACGGGATTGCTTACTGG 360
S A V Y Y C T S R S T M I T T G F A Y W 120
CDR3

GGCCAAGGGACTCTGGTCACTGTCTCTGCAGCTACAACAAACAGCCCCATCTGTCTATCCA 420
G Q G T L V T V S A A T T T A P S V Y P 160

CTGGCC
L A

FIG. 60

AATGACTTCGGGTTGAGCTGGGTTTTATTATTGTTCTTTAAAAGGGTCCGGAGTGAA 60
N D F G L S W V F I I V L L K G V R S E 20

GTGAGGCTTGAGGAGTCTGGAGGAGGCTGGGTGCAACCTGGAGGATCCATGAAACTCTCC 120
V R L E E S G G G W V Q P G G S M K L S 40

TGTGTAGCCTCTGGATTTACTTCAGTAATTACTGGATGACTTGGGTCCGCCAGTCTCCA 180
C V A S G F T F S N Y W M T W V R Q S P 60
CDR1

GAGAAGGGCTTGAGTGGGTTGCTGAAATTGAGATCTGAAAATTATGCAACACAT 240
E K G L E W V A E I R L R S E N Y A T H 80
CDR2

TATGCGGAGTCTGTGAAAGGGAAATTCAACCATCTCAAGAGATGATTCCAGAACGTCTC 300
Y A E S V K G K F T I S R D D S R S R L 100

TACCTGCAAATGAACAACTTAAGACCTGAAGACAGTGGATTATTACTGTACAGATGGT 360
Y L Q M N N L R P E D S G I Y Y C T D G 120

CTGGGACGACCTAACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCCAAACGACA 420
L G R P N W G Q G T L V T V S A A K T T 140
CDR3

CCCCCATCTGTCTATCCACTGGCCCCTTGTGTA
P P S V Y P L A P C V

FIG. 61

CDR1 Comparisons

1G8	1gG _{1k}	Middle	G F N I K D Y Y I H
2H9	1gG _{1k}	N-Term.	G F T F S N Y W M T
4A10	1gG _{2ak}	N-Term.	G Y T F S S Y W M H

CDR2 Comparisons

1G8	1gG _{1k}	W I D P E N G D T E F V P K F Q G
2H9	1gG _{1k}	E I R L R S E N Y A T H Y A E S V K G
4A10	1gG _{2ak}	N I D P G S G Y T N Y A E N L K T

CDR3 Comparisons

1G8	1gG _{1k}	G G F
2H9	1gG _{1k}	L G R P N
4A10	1gG _{2ak}	R S T M I T T G F A Y

FIG. 62

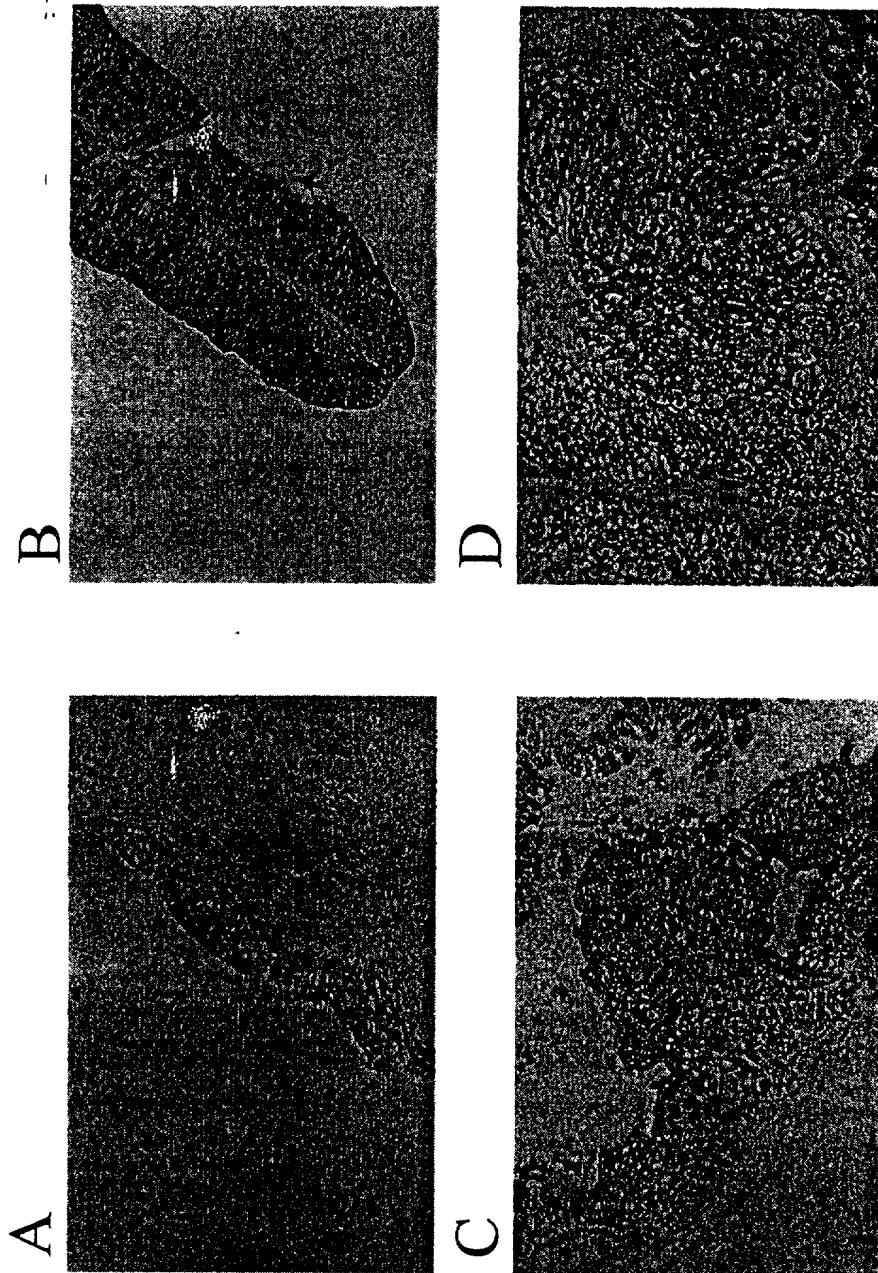
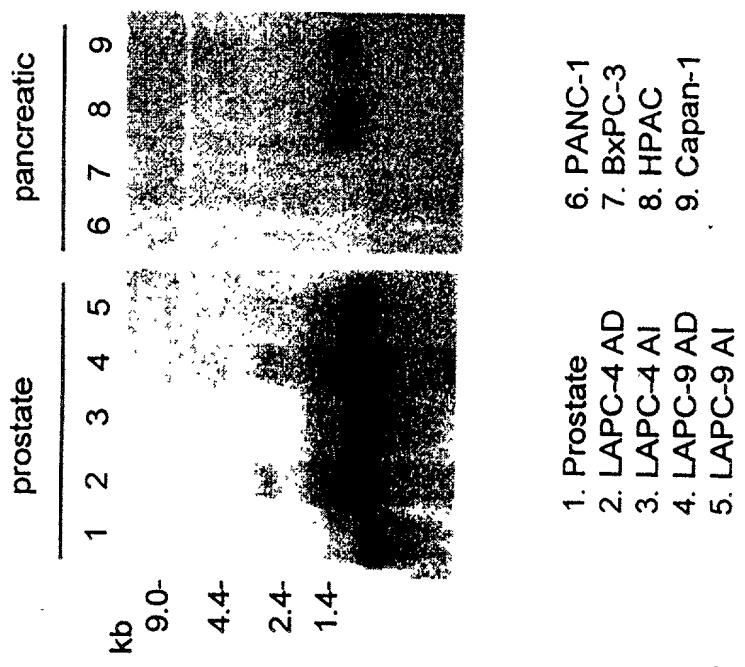
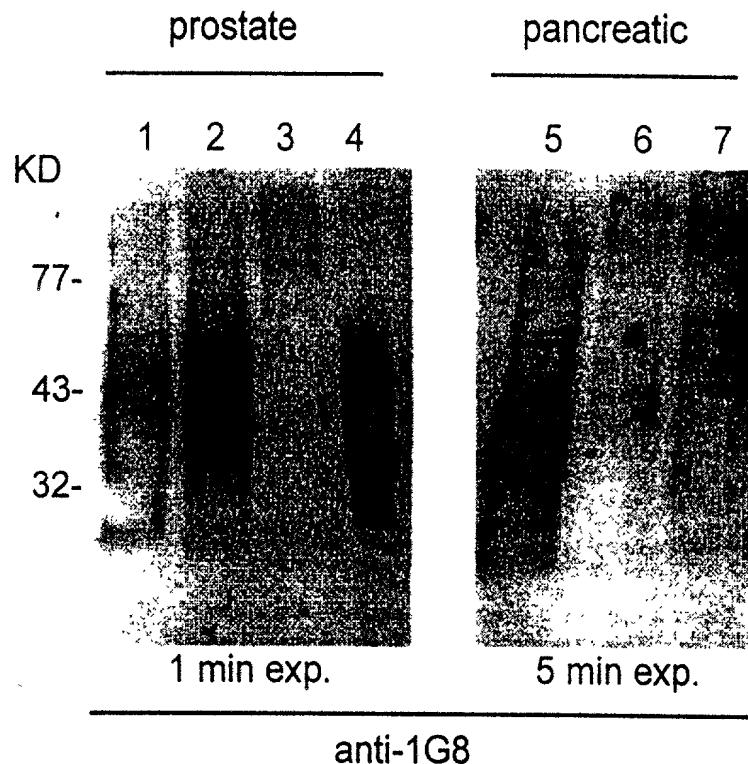


FIG. 63



1. Prostate
2. LAPC-4 AD
3. LAPC-4 AI
4. LAPC-9 AD
5. LAPC-9 AI
6. PANC-1
7. BxPC-3
8. HPAC
9. Capan-1

FIG. 64



1. LAPC-4 AD
2. LAPC-9 AI
3. LNCaP
4. LNCaP-PSCA

5. HPAC
6. Capan-1
7. ASPC-1

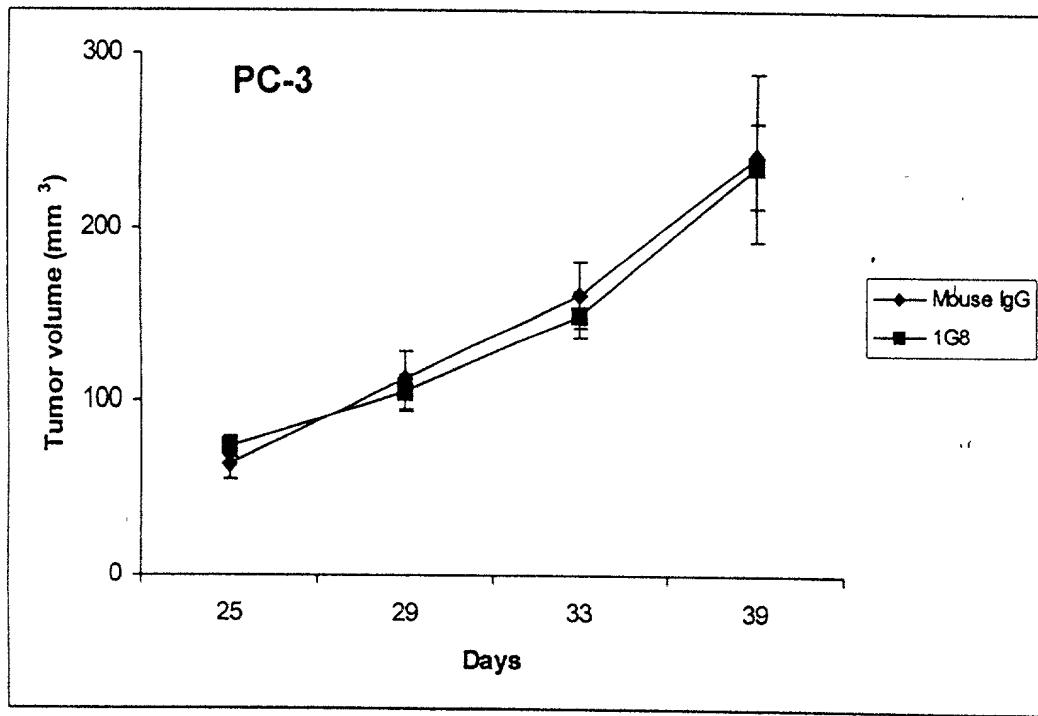
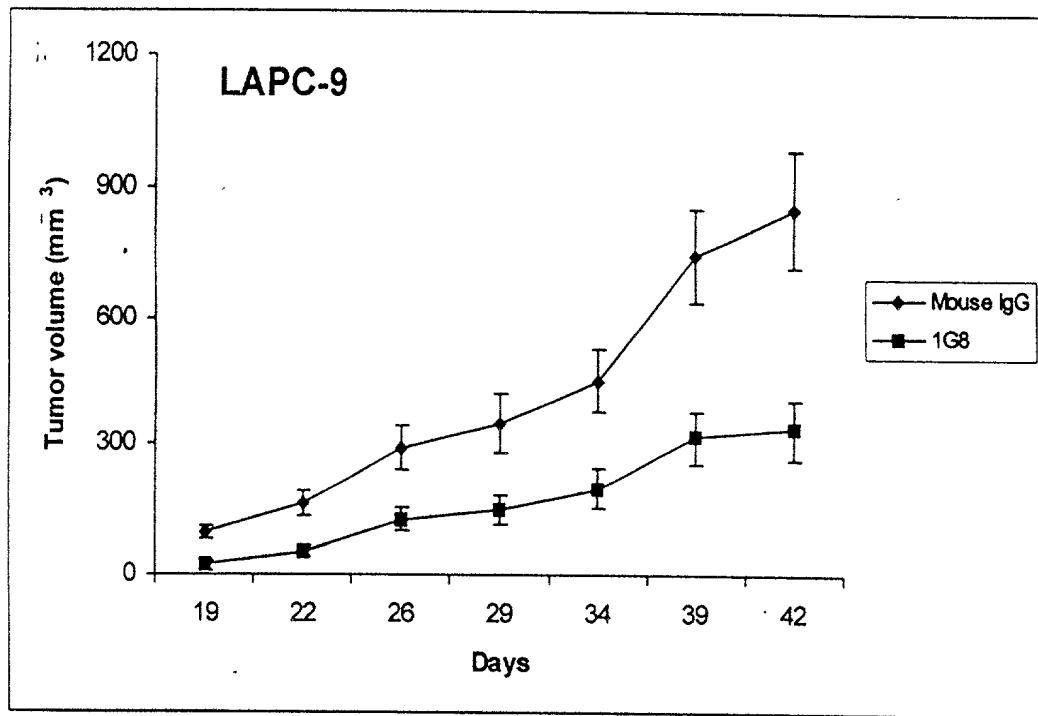


FIGURE 65

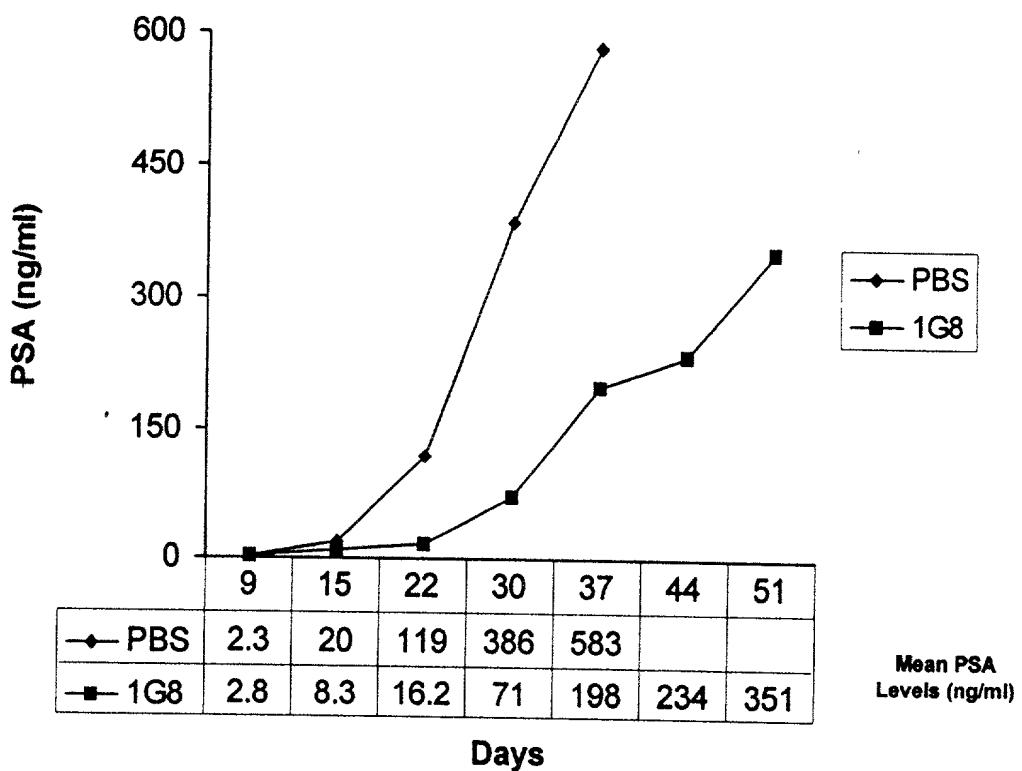
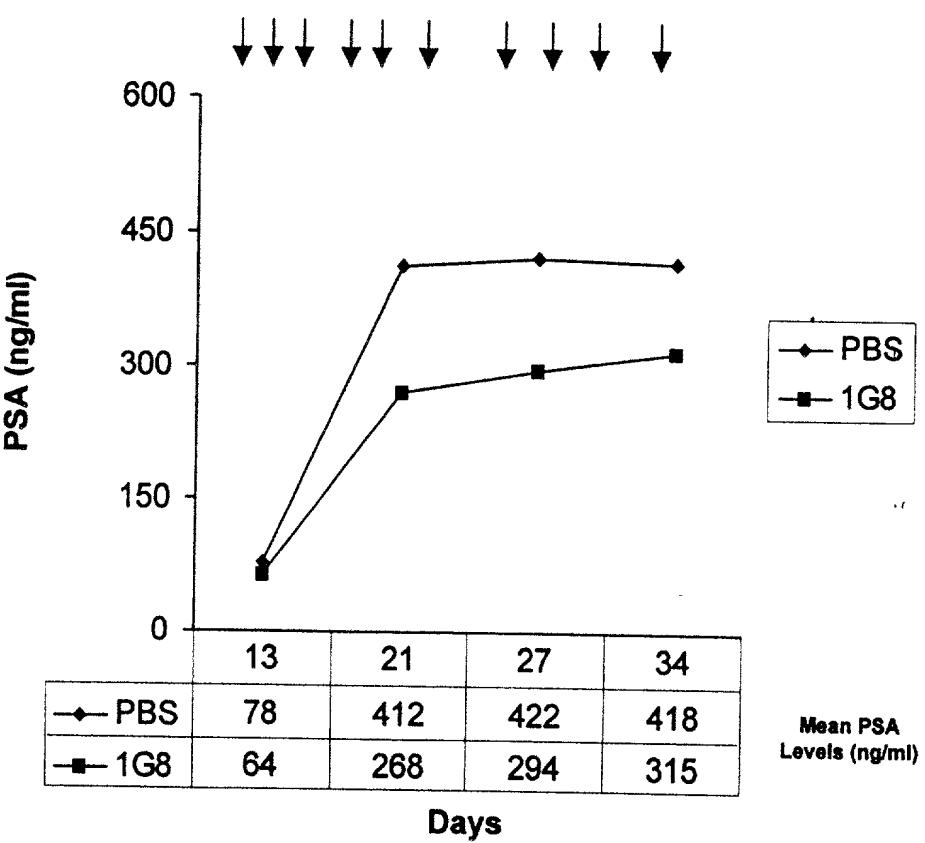
A)**B)**

Figure 66

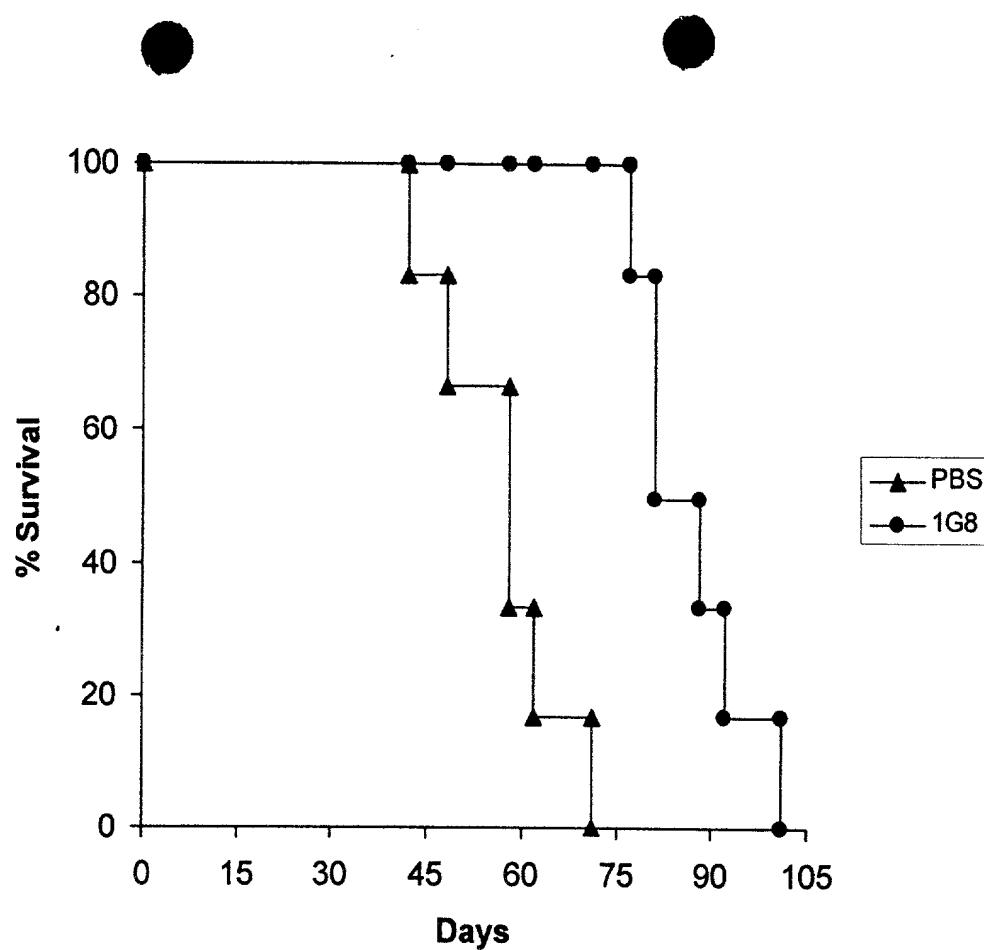
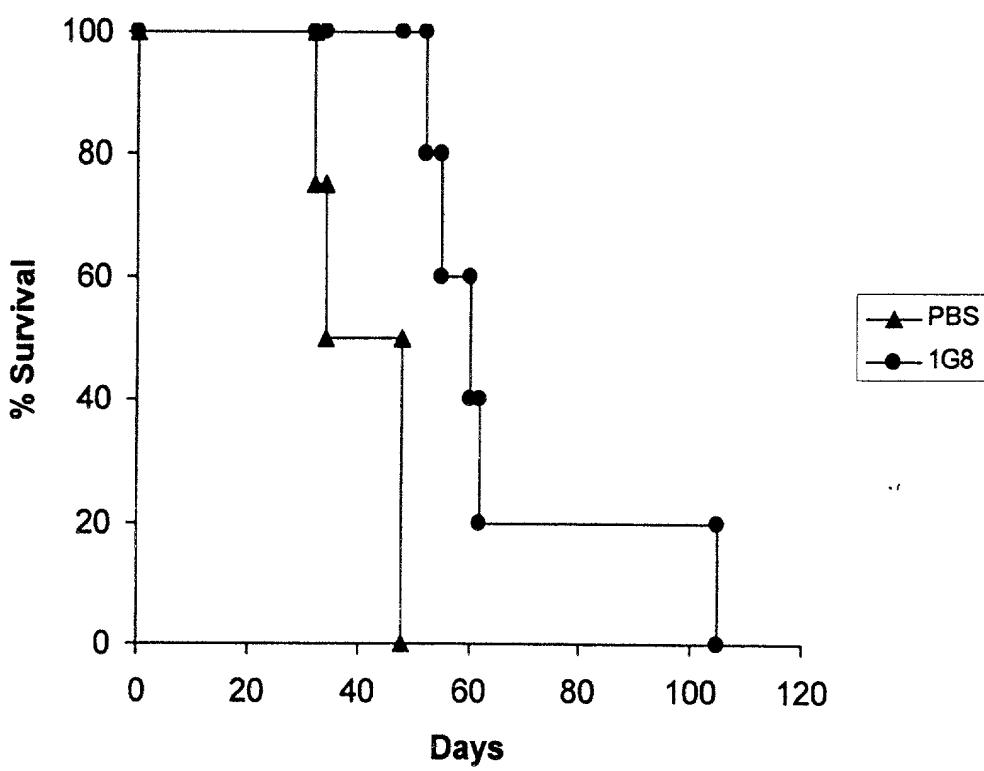
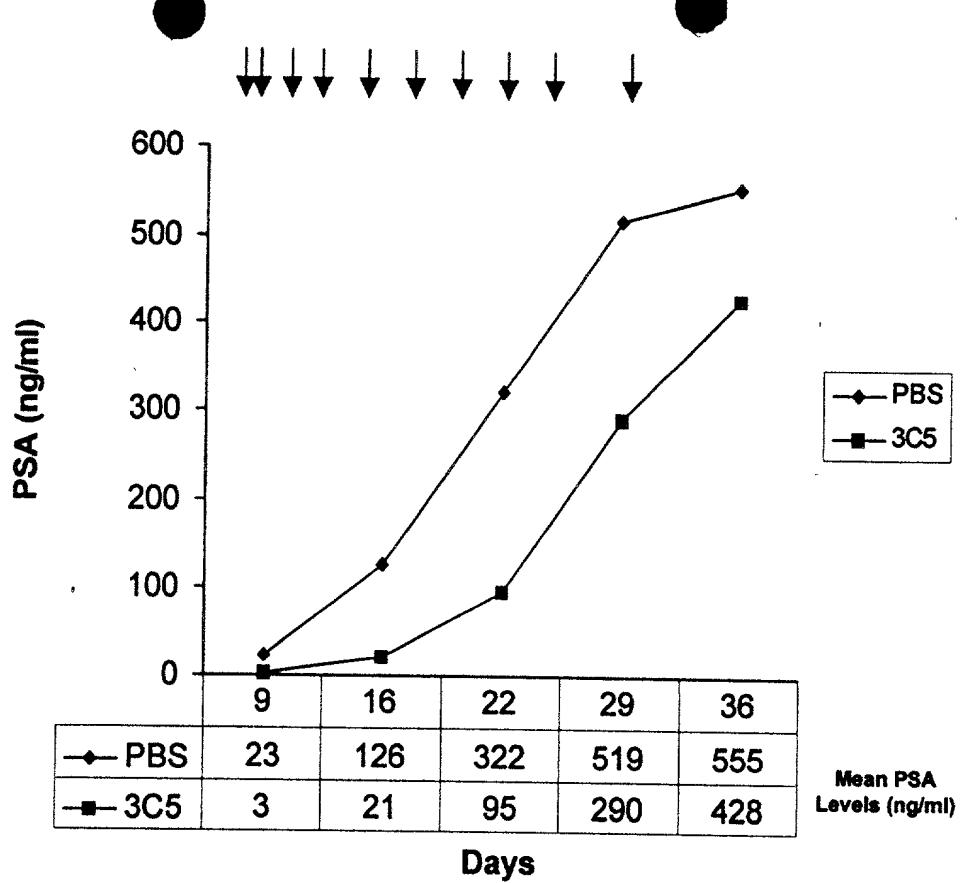
A)**B)**

Figure 67

A)



B)

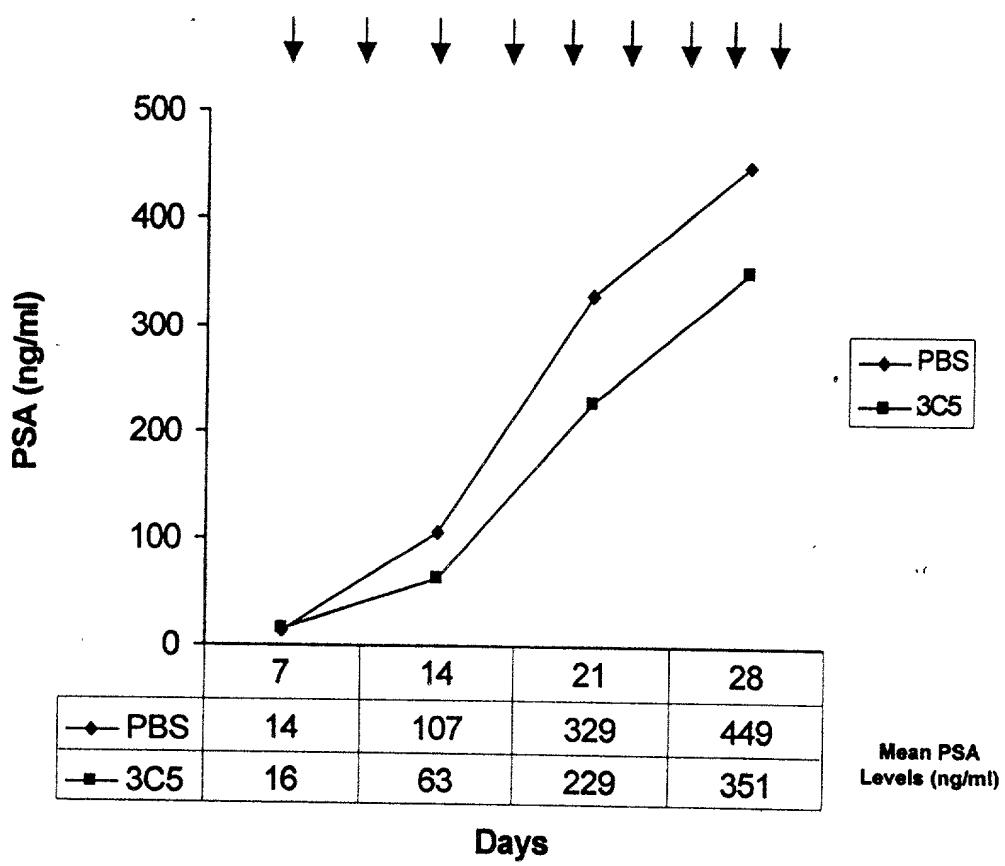


Figure 68

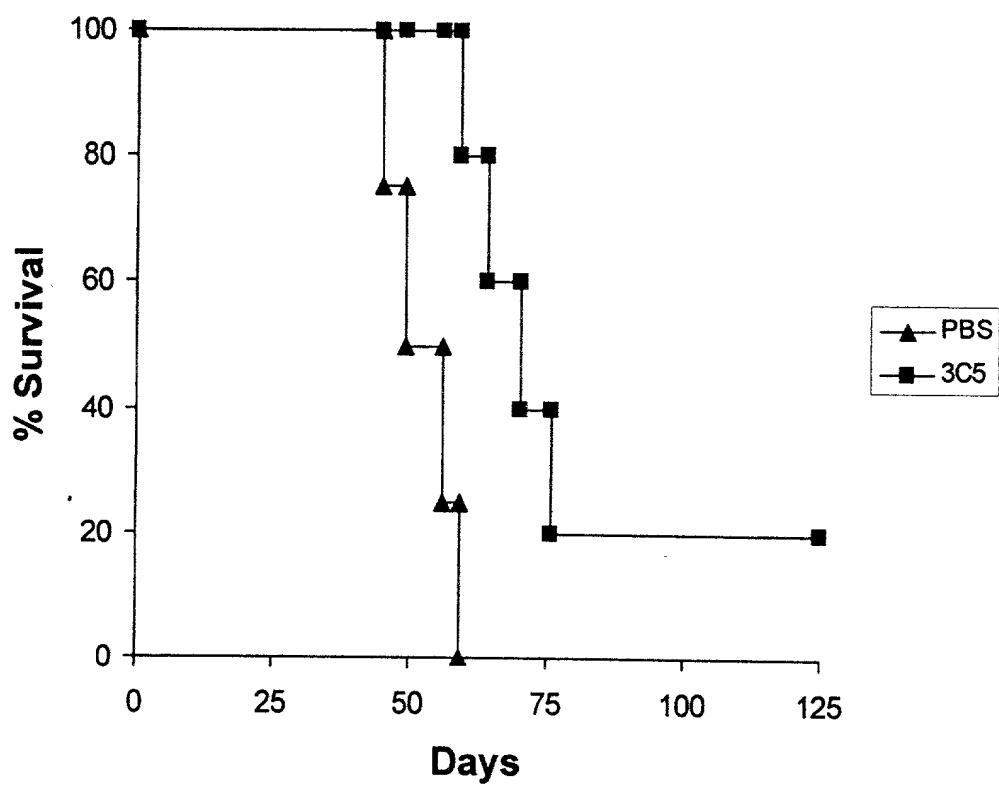
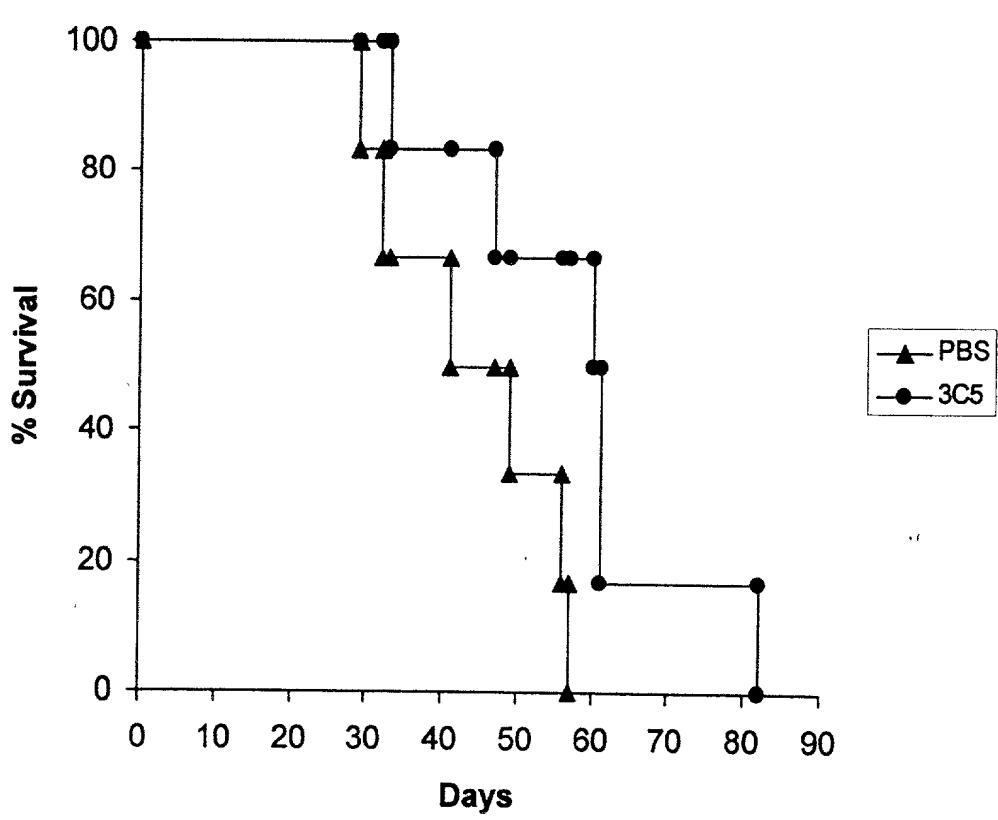
A)**B)**

Figure 69

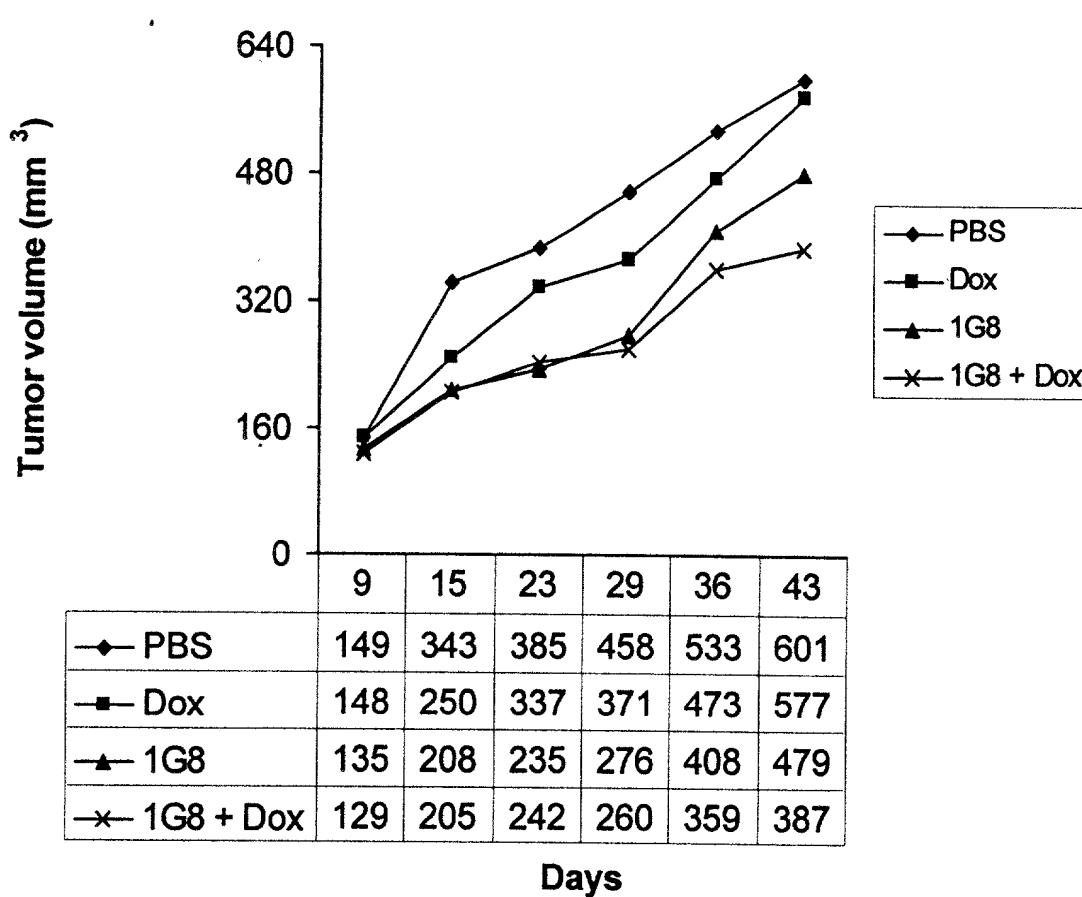
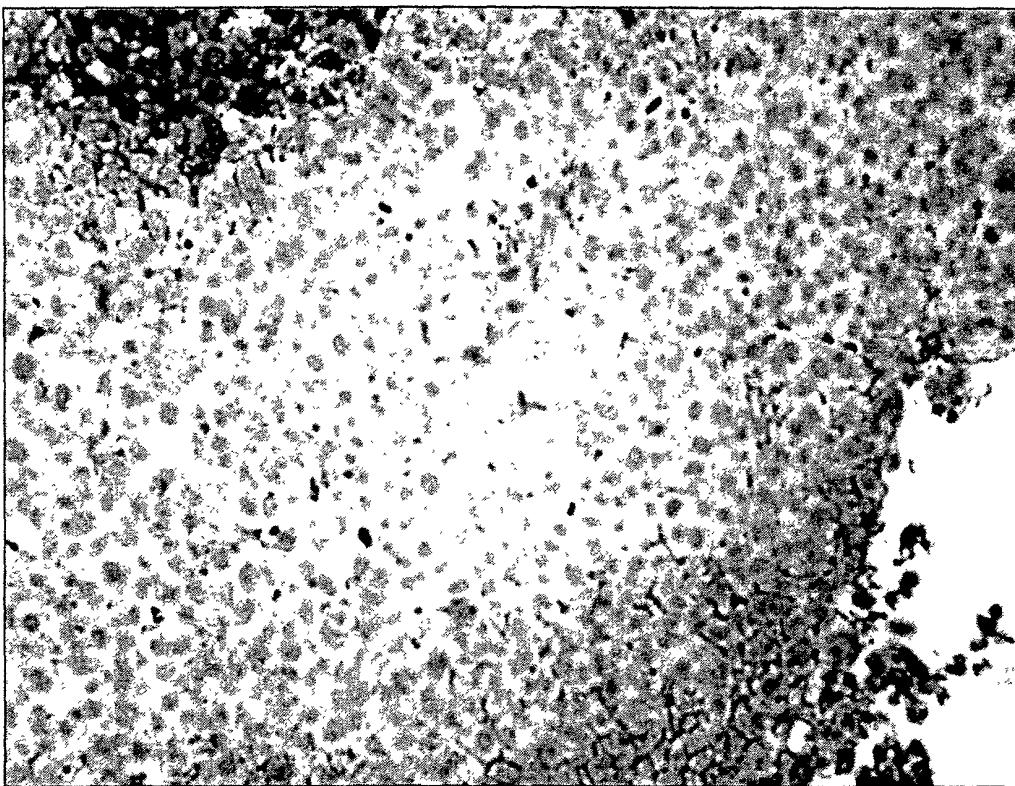


Figure 70

PSCA 3C5 MAb Localizes within LAPC9AD Xenograft Tissue

3C5 Treated



mIgG Treated

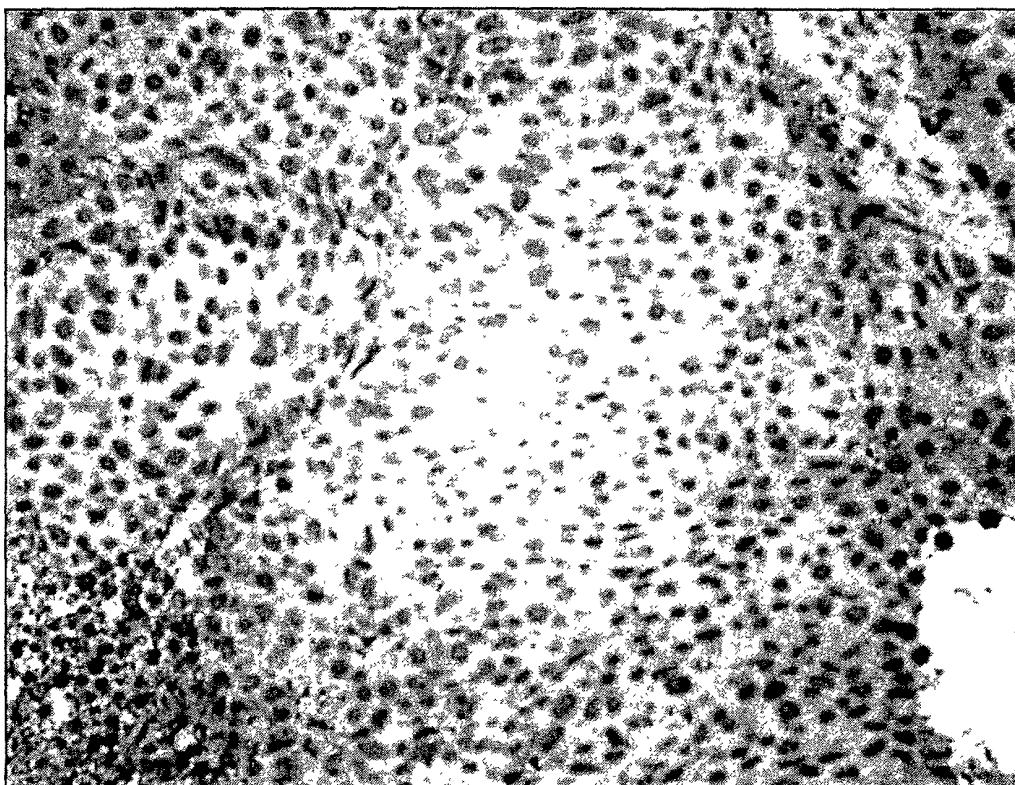
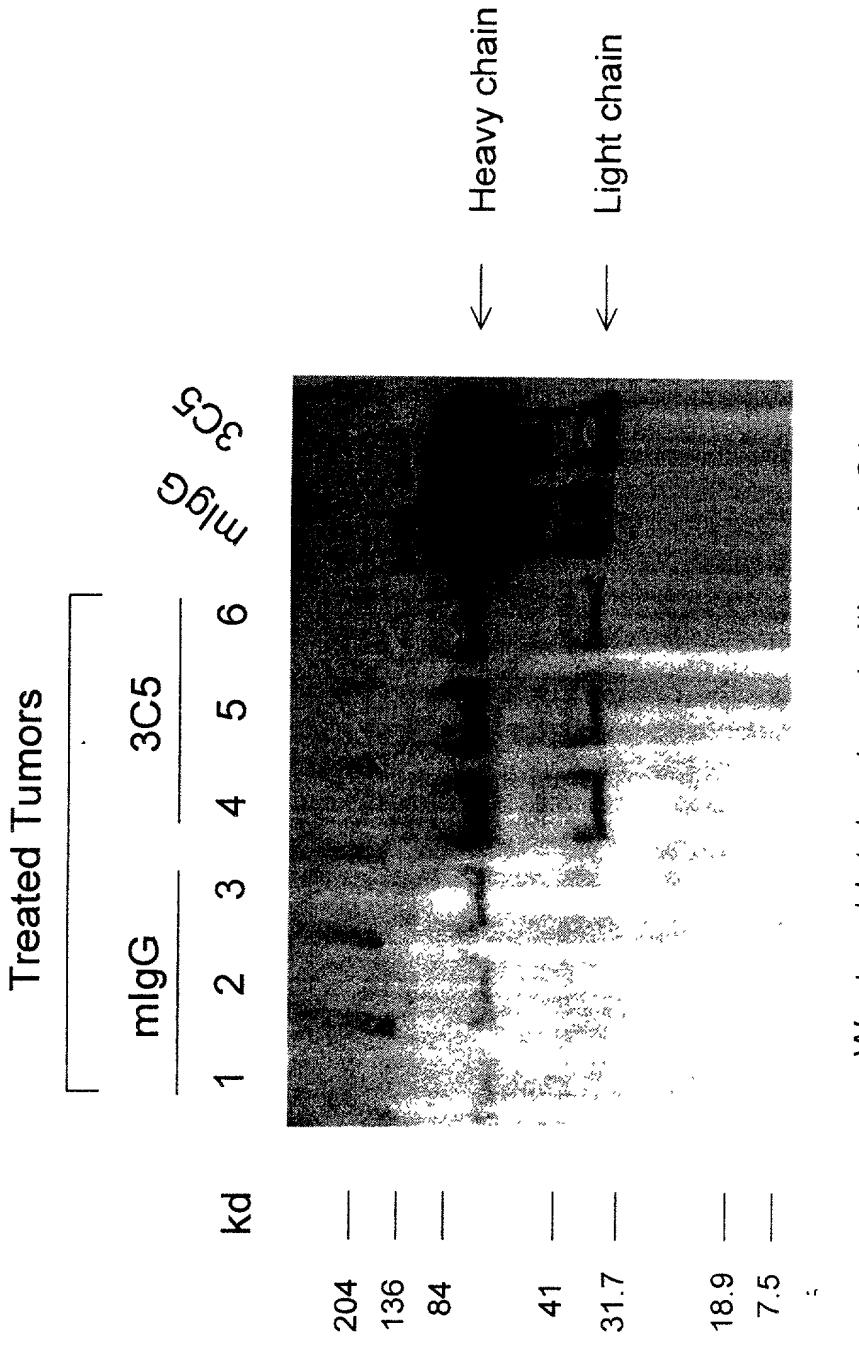


Figure 71

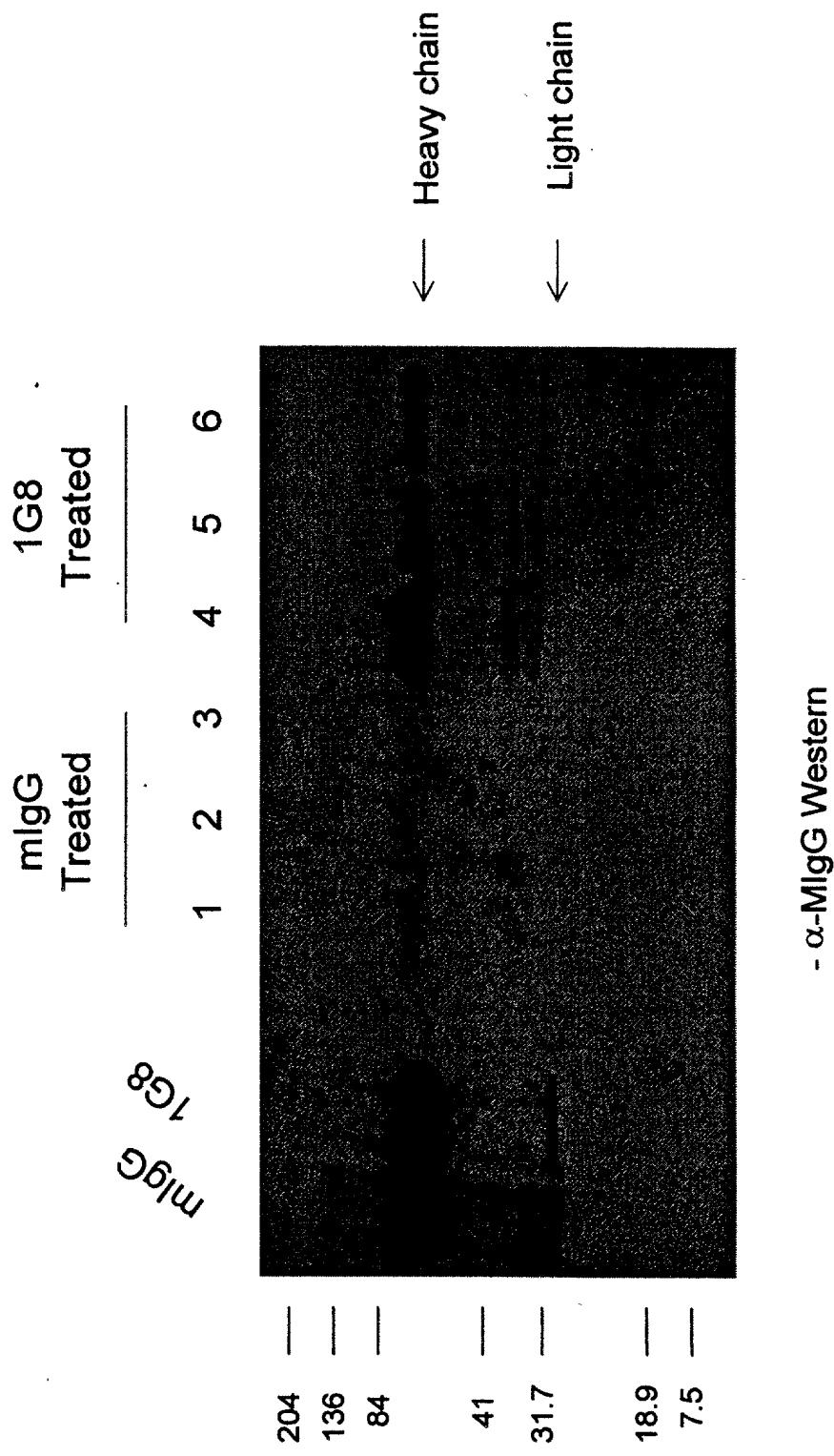
3C5 Anti-PSCA MAb is Localized to Established LAPC-9 Tumors



Western blot developed with α -mIgG/ κ

Figure 72

SPECIFIC TARGETING OF THE 1G8 ANTI-PSCA MAb TO ESTABLISHED LAPC-9 TUMORS



Method: Mice bearing established LAPC-9 tumors (>100 mm³) were injected with either mIgG or the anti-PSCA MAb 1G8. Tumors were harvested a week later and made into protein lysates for Western analysis.

Figure 73